# Increasing the success of community transfer when creating species-rich meadows using green hay strewing

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This work or any part thereof has not previously been presented in any form to the University or to any other body (unless otherwise indicated). Save for any express acknowledgements, references and/or bibliographies cited in the work, I confirm that the intellectual content of the work is the result of my own efforts and of no other person.



Signature: A. Wilkes

Date: 31.3.2017

*Said a traveller by the way*
*Pausing, "What hast thou to say,*
*Flower by the dusty road,*
*That would ease a mortal's load?"*

Traveller, hearken unto me!
I will tell thee how to see
Beauties in the earth and sky
Hidden from the careless eye.
I will tell thee how to hear
Nature's music wild and clear

*Bliss Carman*

To see a World in a Grain of Sand
And a Heaven in a Wild Flower,
Hold Infinity in the palm of your hand
And Eternity in an hour

*William Blake*

Many eyes go through the meadow, but few see the flowers in it.

*Ralph Waldo Emerson*

Flowers always make people better, happier, and more helpful;
they are sunshine, food and medicine for the soul.

*Luther Burbank*

Just living is not enough... one must have sunshine, freedom, and a little flower.

*Hans Christian Andersen*

*For memories of days spent surveying:*

I'm just sitting watching flowers in the rain.

*The Move (Song lyric)*

*And finally, just for fun:*

If the English language made any sense, lackadaisical would
have something to do with a shortage of flowers.

*Doug Larson*

## Abstract

Methods of increasing the number, diversity and evenness of plant species establishing in species-rich meadows created or enhanced with green hay from a semi-natural source meadow were studied. Three experiments were conducted on grasslands in Birmingham and Herefordshire: (i) Comparisons of species and community transfer resulting from green hay being strewn in consecutive years onto a glyphosated receiver meadow. (ii) The effect on species-richness of introducing green hay into a species-rich created meadow. (iii) The effect of different levels of disturbance in combination with grazing on the introduction of species into an existing created species-rich meadow.

Strewing hay twice resulted in vegetation containing more species and species with higher frequencies compared with haying once. Hay strewing increased the number of species in an existing species-rich sward and also increased the frequency and abundance of existing species. Source species frequency, flowering/seed set date and established life strategy had an important influence on species transfer. In general, species that did not transfer were those found at low frequencies in MG5 *Cynosurus cristatus – Centaurea nigra* community meadows and with stress-tolerance as part of their life strategy. There was a statistically significant three-way interaction between haying, grazing and disturbance.

As several terrestrial orchid species are associated with this habitat type, techniques and media for axenic seed germination and propagation of a selected local MG5 meadow orchid species (*Dactylorhiza fuchsii*) were assessed. Comparisons were made of two media types in combination with mycorrhizae and a source of complex carbohydrates. Of these, oats medium with fungi produced significantly higher germination rates than other tested media. Oats medium also proved the most suitable medium for protocorms when replated, producing the greatest increase in protocorm length compared with Western medium after 15 weeks of growth.

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# Chapter 1

# Introduction

## 1.1 Semi-natural grasslands – the decline in species-rich grasslands

Species-rich grasslands (of which there are several different community types; Rodwell, 1992) are of high conservation value due to their biodiversity (Pywell *et al.,* 2004). However, agricultural intensification, drainage, conversion into cropland, tree planting and abandonment have all led to their decline, in extent and diversity, throughout continental Europe (Stoate *et al.,* 2001; Poschlod and Wallis DeVries, 2002; Stoate *et al.,* 2009) and the United Kingdom (Fuller, 1987; Blackstock *et al.,* 1999; Bullock *et al.,* 2011a, b). It has been estimated that only 3% of the UK's historic species-rich grassland remains (Fuller, 1987; Blackstock *et al.*, 1999; Fuller *et al.,* 2002), with intensively managed, species-poor agricultural pasture now the dominant grassland type across Europe (Pywell *et al.,* 2007) and covering 24% of all of the land in the UK (Fuller *et al.,* 2002), although this loss has now slowed considerably (Bullock *et al.,* 2011b). This loss of grassland habitat has been accompanied by a decline in both flora (Rich and Woodruff, 1996) and fauna including bees (Goulson *et al.*, 2005), other invertebrates (Littlewood *et al.*, 2012; Woodcock *et al.*, 2012a,b) and birds (Vickery *et al.,* 2001) and has also led to the isolation of the remaining examples of the habitat (Poschlod *et al.,* 1998; Blackstock *et al.*, 1999; Ruprecht, 2006). This isolation has resulted in a reduction in the dispersal of propagules between plant populations, which previously occurred through biotic (including via the effect of and the movement of livestock) and abiotic dispersal, transport of hay (Hedberg and Kotowski, 2010) and sharing of harvesting machinery (Wallin *et al.,* 2009).

## 1.2 The MG5 *Cynosurus cristatus-Centaurea nigra* community and the current status of the habitat in the UK

Grassland habitats include a range of vegetation types which, in the UK, have been categorised using the National Vegetation Classification (Rodwell, 1992). There are thirteen mesotrophic grassland (MG) communities, fourteen calcicolous grasslands (CG) and seven calcifuge grasslands (U; Rodwell, 1992). The MG5 *Cynosurus cristatus-Centaurea nigra* community, upon which this study is based, is a species-rich mesotrophic grassland (with an average of 23 species per 4 $m^2$), on neutral soil which is characteristically managed as a traditional hay meadow (Rodwell, 1992). For the NVC, 194 of the MG5 meadows in the UK were surveyed (Figure 1.1; Rodwell, 1992). This plant community is found throughout the UK, in lowland areas, but its extent has been severely reduced by changes in agricultural practises (Blackstock *et al.* 1999; Fuller 1987). It is a variable community, in both species and appearance, often due to differences in soil conditions between and within individual meadows (Rodwell, 1992). There are three sub-communities: MG5a *Lathyrus pratensis,* MG5b *Galium verum* and MG5c *Danthonia decumbens* and the differences between them are mainly related to variations in soil conditions, i.e. pH, calcium content, trophic conditions and moisture content (Rodwell, 1992).

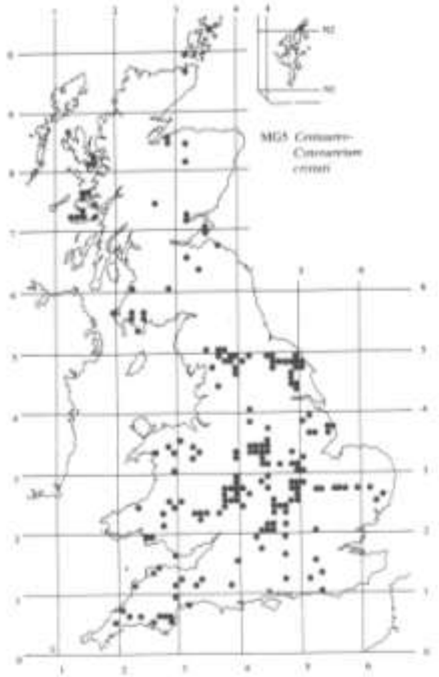

Figure 1.1: Map of the MG5 *Cynosurus cristatus-Centaurea nigra* community meadows surveyed for the NVC, (p. 66; Rodwell, 1992).

Seventy percent of semi-natural, neutral, dry, species-rich grassland in England and Wales is of the MG5 community type, which is estimated to total only 5,000-10,000 ha (Blackstock *et al.,* 1999). However, even this figure may be an overestimate, as the condition of some sites is declining or poor. For example, O'Reilly (2010) reports on the findings of surveys which re-visited meadows assessed to be of high quality in the mid-1980s. Twenty years later, only 20% of these sites retained this high quality. Similarly, of the 1602.5 ha of neutral grassland Sites of Special Scientific Interest (SSSIs) covered by the West Midlands Region of Natural England (NE), 52.5% are in a favourable condition and the remainder are in an unfavourable condition, although 40.1% are recovering (Figure 1.2; NE, 2013). The England Biodiversity Strategy targets include the commitment that by 2020, at least

50% of SSSIs should be in a favourable condition and at least 95% should be in a favourable or recovering condition (HM Government, 2011); also, more relevant to habitat creation, that these are part of a joined-up network of large, good quality habitats (Lawton *et al.,* 2010; HM Government, 2011; Chaplin, 2012).

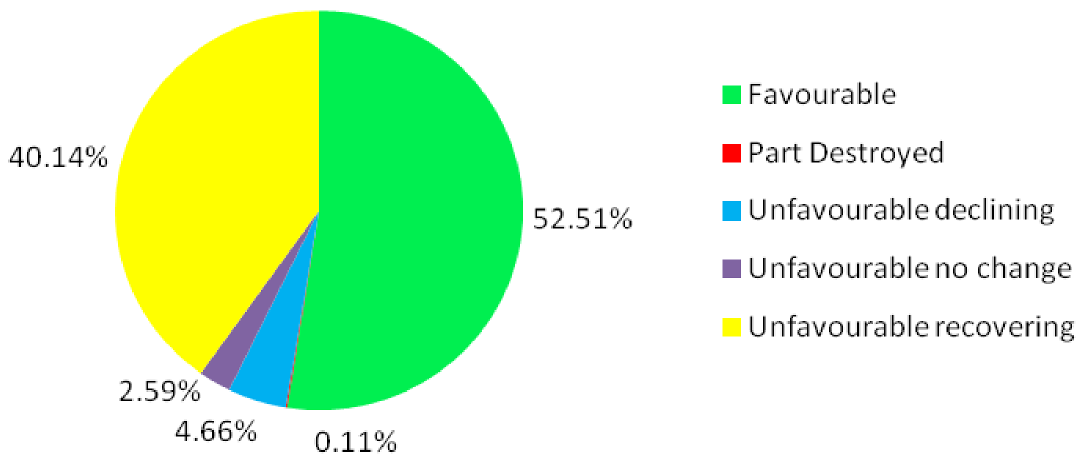


Figure 1.2: Condition of Neutral Grassland SSSI Units in the West Midlands NE Team (by area; NE, 2013); data last compiled 1/8/12.

### 1.3 The development of grassland creation, enhancement and restoration projects and the need for such projects to improve their outcomes

The conservation and restoration of species-rich grasslands has been an important objective in nature conservation since the 1970s (Kiehl *et al.,* 2010). Grassland 'creation' and 'restoration' are not always defined in the literature, although there are a number of articles that do define these terms (e.g. Anderson, 1995; Walker *et al.,* 2004; SER 2004). These articles suggest the use of 'restoration' for sites where remnants of the habitat/community still exist (e.g. agriculturally semi-improved grassland) and 're-creation' or 'creation' for sites where the target community was present in the past, but the land-use has changed (e.g. arable land).

In the UK, creation and restoration projects include those led by science-based research groups, for example, the Open University Floodplain Meadows Partnership; the River Ouse Project which includes the University of Sussex, the University of Reading and the Native Seed Hub Project at Kew. There are also numerous community projects and projects led by conservation organisations, farms and government departments, which have a variety of funding sources including agri-environment schemes and charitable trusts. Grassland restoration and creation is the most common task funded by agri-environment schemes (Anon., 2009; Pywell *et al.*, 2012) and is also one of the most costly (Woodcock *et al.,* 2012a; Hewins *et al.*, 2012). The number of projects and the variety and number of people and organisations involved indicates the amount of money, time and effort spent on species-rich grassland creation and restoration projects. This emphasizes the need for rigorous scientific research to provide the knowledge to inform and underpin such projects in future in order to increase their success and to maintain public interest, funding and protection for these habitats (Kiehl, 2010; Hewins *et al.*, 2012). The current economic situation and potential changes in the Common Agricultural Policy (CAP) also mean that more successful and efficient projects and schemes are needed, due to the potential reduction in funding (Chaplin, 2012). Brexit adds to this situation, as it also may lead to changes in funding. Conversely, publications such as the Lawton Review (Lawton *et al.,* 2010), the National Ecosystem Assessment (Watson and Albon, 2011) and the Natural Environment White Paper (DEFRA, 2011) set out challenging targets for protecting and increasing biodiversity (Chaplin, 2012), suggesting a need for more funding. For example, the Biodiversity Strategy includes the target of increasing the overall extent of priority habitats by at least 200,000 ha by 2020 (HM Government, 2011).

## 1.4 Conservation techniques used in the creation, enhancement and restoration of species-rich grassland – the form of the introduced material

The aim of the first series of species-rich grassland creation and restoration projects was often to improve the site conditions to allow for natural regeneration/ colonisation of the habitat (Kiehl *et al.,* 2010). However, a lack of seed rain of appropriate species due to the isolation of sites (Mortimer *et al.*, 1998; Bakker and Berendse, 1999; Pywell *et al.,* 2002), the limited dispersal distances of meadow species (Donath *et al.,* 2003; Bischoff, 2000; Coulson *et al.,* 2001; Poschlod *et al.,* 1998) and the lack of a persistent seed bank for the majority of these species (Grime *et al.,* 1988; Bakker *et al.,* 1996; Mitlacher *et al.*, 2002; Bossuyt and Honnay, 2008) led to the conclusion that, in most cases, natural colonisation was unlikely. This means that the target species need to be added to sites to facilitate the creation of these habitats (Wells, 1989; Bakker *et al.,* 1996; Coulson *et al.,* 2001; Walker *et al.,* 2004; Holzel, 2012).

A number of techniques to introduce the target plant species to project sites have been tested relating to the form of the introduced material. These include: the transfer of turves and seed-containing soil (e.g. Good *et al.,* 1999; Vecrin and Muller, 2003; Cobbaert *et al.,* 2004; Trueman *et al.,* 2007), the use of plug plants (e.g. Davies *et al.,* 1999; Hopkins *et al.,* 1999; Brown and Bugg, 2001; Huddleston and Young, 2004; Wallin *et al.,* 2009), the use of commercially bought seed mixtures, which can be introduced by simple scattering or by more involved techniques, such as slot seeding (Wells, 1989; Hopkins *et al.,* 1999), hay strewing (Jones *et al.,* 1995; Trueman and Millett, 2003; Donath *et al.,* 2007; Edwards *et al.,* 2007; Rydgren *et al.,* 2010, Cornish and Hooley, 2012; Starr-Keddle and Barrett, 2012; Kirkham *et al*., 2013), brush harvested seed (Edwards *et al.,* 2007; Scotton *et al.,* 2009), vacuum harvested seed (Riley *et al.,* 2004; Stevenson *et al.,* 1997) and transfer of litter from existing meadows (Stroh *et al.,* 2002).

In a survey of 81 grassland projects, Stevens and Wilson (2012) found that four major methods were used: commercially bought seed mixes (20 sites), locally sourced seed (either from a local SSSI or reputable seed supplier: harvesting method not stated) (24 sites), green hay strewing (12 sites) and natural regeneration (12 sites, near a species-rich source site). Each creation method has advantages and disadvantages (Jongepierova *et al.,* 2007;

Stevens and Wilson, 2012). The transplantation of turf is likely to be most appropriate when the source site is being destroyed (e.g. through construction work), that is, where the primary aim is to translocate a community rather than to create a habitat, because, by definition, the removal of turves damages the source habitat. Plug plants are expensive per plant compared to seed and studies using plug plants have shown variable success (e.g. Morgan, 1999; Huddleston and Young, 2004; Wallin *et al.,* 2009; Sprunger and Prendergest, 2010). Therefore, especially on large sites, plug plants are most likely to be used to introduce species that are hard to obtain as seed, or are difficult to establish from seed (Wells, 1989; Hopkins *et al.,* 1999; Morgan, 1999; Pakeman *et al.,* 2002; Christian and Peel, 2009; Huddleston and Young, 2004; Jongepierova *et al.,* 2007; Wallin *et al.,* 2009). This includes orchid species (Ramsay and Stewart, 1998; Batty *et al.,* 2006; Scade *et al.,* 2006; Kauth *et al.,* 2010; Sprunger and Prendergest, 2010). Introduction and re-introduction projects involving orchids are discussed further in Section 1.8 and Chapter 6. Conversely, the use of seed, from whatever source, is more likely to be suitable in a wider range of grassland creation projects.

## 1.5 The use of seed: implications and the importance of local provenance

In a review of grassland creation projects across the world (the majority of which were in Europe), Hedberg and Kotowski (2010) found that seed (as seed mixes, or in hay or vegetation litter) was the most commonly used form in which to introduce target species to a study/project site. This was most often through scattering of seed (e.g. using a commercially bought seed mixture) rather than through slot seeding, hay strewing, the use of brush harvested seed or transfer of litter (Parker, 1995; Hedberg and Kotowski, 2010), perhaps due to the ease and familiarity of the use of commercially bought seed (Hedberg and Kotowski, 2010). However, there are a number of problems associated with the use of commercially available seed, such as non-local and unsuitable provenance (e.g. Auestad *et al.*, 2015), poor and/or lengthy storage (and consequent potentially poor viability), lack of availability of seed of rare plants and the creation of unnatural communities, in terms of the species mix and/or the proportion of species. It is also important for individual meadow creation projects to take into account the composition of their local grasslands, especially given the spectrum of semi-natural meadow communities in the UK (Parker, 1995; Rodwell, 1992), otherwise regional variations in grassland types could be lost (Wagner *et al.*, 2012; Stevens and Wilson, 2012). This variation may be more difficult to create when using commercially bought seed compared to techniques which source seed 'mixes' locally, for example, techniques such as hay strewing. Heterogeneity, across and within habitats and sites, is also thought to be of key importance when aiming to support a high number of species (Webb *et al.*, 2010; Radley, 2012), which is a central aim of the Biodiversity 2020 initiative (DEFRA, 2011). Some companies sell seed mixtures as harvested from existing meadows (Flora Locale, 2016) although the number of sites harvested from is limited and so may not be very local to an individual creation site.

The use of local provenance seed is important to preserve local genetic variation (Jones and Hayes, 1999; Sackville-Hamilton, 2001; Bischoff *et al.,* 2010) and also to improve the likelihood of fitness for the local conditions, thereby increasing the chance of creation success (Smith *et al.,* 2005; Bischoff *et al.,* 2006; Zeiter and Stampfli, 2008; Smith *et al.,* 2009; Vander Mijnsbrugge *et al*., 2010) and conversely to prevent the introduction of aggressive alien genotypes (Jones and Hayes, 1999; Bischoff *et al.,* 2006; Bischoff *et al.,* 2010; Vander Mijnsbrugge *et al*., 2010). Preservation of local genetic variation is important to preserve genetic diversity in the overall population, to increase the chance of fitness for a change in conditions (Jones and Hayes, 1999; Hughes and Stachowicz, 2004; Reusch *et al.,* 2005). The use of local provenance seed is also important due to effects such as outbreeding depression, which can occur through hybridization of local and non-local populations and also when seed mixes contain seeds from a number of different non-mixing populations which may threaten the long-term stability of the created communities (Eckstein and Otte, 2005). Keller *et al.* (2000) found a negative effect of outbreeding when crossing foreign sourced seed with local provenance seed. There may also be consequences for invertebrate herbivores (Keller *et al.,* 1999; Smith, 2007) e.g. through effects on larval growth (Smith, 2007). Both ecological provenance (i.e. habitat similarity) and geographical provenance are important (Walker *et al.,* 2004; Smith *et al.,* 2005; Bischoff *et al.,* 2006; Smith *et al.,* 2009) due to the genetic differences between populations from different regions and the differing conditions prevalent in different habitats.

This includes subtle differences, such as the timing of the hay cut and the consequent effect on the timing of seed set (Eriksson *et al.,* 2015; Reisch and Poschlod 2009; van Andel, 1998; Smith and Jones, 1991). A conflicting view is that inbred, genetically similar, isolated populations may need the introduction of unrelated populations to increase their chance of survival if faced with changes in their environment (Kaye, 2001; Vergeer *et al.,* 2004). Keller and Waller (2002) stated that (at the time of publication) there was more evidence for the negative effects of inbreeding than for outbreeding, although Vander Mijnsbrugge *et al*. (2010) point out that outbreeding effects can be harder to evaluate. In the collection of seed for creation projects, the size of the sampled population is also important as smaller, isolated populations can be more inbred and thus less genetically diverse (Vander Mijnsbrugge *et al*., 2010).

## 1.6 The current status of grassland creation research

The majority of studies generally conclude that species-rich grassland creation is feasible and worthwhile, at least with regard to the botanical community (Anderson, 1995; Dobson *et al.,* 1997; Manchester *et al.,* 1999; Walker *et al.,* 2004; Kiehl *et al.,* 2010; Hedberg and Kotowski, 2010; Piqueray and Mahy, 2010). Individual projects may fail, for a small number of reasons, namely poor planning, objectives that were too ambitious, lack of soil testing or ignoring the results of soil tests, the need for site management not being taken into account and inadequate site monitoring (Parker, 1995). Hedberg and Kotowski (2010) suggested that the most appropriate technique depends on the actual project (because of financial and/or logistical considerations) and the characteristics of the target species (e.g. germination and establishment traits and species rarity). When comparing vacuum harvesting and hand collection, Stevenson *et al.* (1997) recommended that the best collection technique for collecting seed of a particular species depends on the species and the site. However, Parker (1995) noted a need for more publication of case studies of grassland creation projects, particularly those that fail or are less successful, so that the knowledge gained from these projects is disseminated. Hedberg and Kotowski (2010) also consider that publications related to grassland creation could be improved by reporting on species that fail, or only reach low levels of abundance to increase the knowledge and information available on these species. Existing literature suggests that this has yet to become normal practise, although some papers do report on all species (e.g. Pywell *et al.*, 2003). It can also be difficult to assess 'success' due to problems with experimental design and the different methods of assessing success used in the literature, as well as the decision regarding at what threshold of what measure is 'success' achieved (e.g. White and Walker, 1997; Ruiz-Jaen and Aide, 2005; Ehrlen *et al.,* 2006; Ruprecht, 2006).

Kiehl *et al.* (2010) and Rydgren *et al.* (2010) point out the difficulty in comparing studies (and therefore creation methods) due to inconsistency in experimental design, in methods used to compare project and reference sites, in data analysis and in data presentation. They suggest, therefore, the need for experiments that are more comparable. In addition, in the UK and across Europe, there are many different meadow types and different methods may be applicable to these different types, or at least, different factors may affect different meadow types, meaning that experiments on similar communities are more comparable than those between communities.

Grassland creation research continues to test initial creation techniques, such as natural regeneration (Lencova and Prach, 2011), seed sowing (Oster *et al.,* 2009; Wallin *et al.,* 2009) and hay strewing (Rydgren *et al.,* 2010; Scotton *et al.,* 2010). For example, Conrad and Tischew (2011) studied grassland creation on arable land when used as a compensation measure for construction projects, concluding that although the current practise was to use uniform commercial seed mixes, it was not an effective way to facilitate the creation of local grassland communities. This supports previous conclusions from Jones and Hayes (1999), Pywell *et al.* (2003) and Walker *et al*. (2004). Eichberg *et al.* (2010) found that topsoil removal with green hay strewing was a successful method to create new sites of endangered calcareous sandy grassland communities.

Other literature investigates, in more detail, factors that are important in grassland creation, such as management (Hejcman *et al.,* 2010; Torok *et al.,* 2010), soil fertility and pH (Kardol *et al.,* 2008; Klimkowska *et al.,* 2010a), soil fauna and microbial communities (Smith *et al.,* 2008; Kardol *et al.,* 2009; De Deyn *et al.,* 2011), plant pathogens (Allan *et al.,* 2010), dispersal

(Bischoff *et al.,* 2009; Klimes *et al.,* 2010), herbivory (Klimkowska *et al.,* 2010b; Pywell *et al.,* 2007), plant traits (Rehounkova and Prach 2010), gap creation and microsite availability (Ruprecht *et al.,* 2010), the meaning of local provenance (Malaval *et al.,* 2010; Mitchley 2010; Seifer and Fischer 2010) and 'community assembly' and succession (Ejrnaes *et al.,* 2006; Bischoff *et al.,* 2009; Rehounkova and Prach 2010). There is also a growing interest in landscape-scale planning of creation projects and the creation of ecological networks against a background of planning for mitigation for climate change and reversing the decline in biodiversity (e.g. Lawton *et al.,* 2012; Trueman *et al.,* 2013). The motivation for grassland creation may also be changing, at least at policy level, from the intrinsic value of re-created habitats to the value that the habitat can provide in terms of ecosystem services (Radley, 2012), such as producing a better quality of meat than improved grasslands (although the evidence for this is inconsistent), as a habitat for UK BAP priority species, as part of the cultural heritage of the UK, for recreation and tourism, as a carbon store and as a source of pollinators and pest control species (Bullock *et al.,* 2011b).

## 1.7 Green hay strewing for species-rich meadow creation and restoration

Hay has been used as a seed source since the times when farmers would re-seed meadows using sweepings from the floor of the hay barn (Losvik and Austad, 2002; Poschlod and Biewer, 2005). However, the use of baled or dried hay had limited success for many species (Wells *et al.,* 1986; Manchester *et al.,* 1999; Trueman and Millett, 2003), either due to reduced viability of the seed (Wells *et al.,* 1986; Manchester *et al.,* 1999; Trueman and Millett, 2003) or through loss of seed through the hay-making process (Trueman and Millett, 2003).

A number of studies have found that green hay strewing is an effective technique for creating and restoring species-rich meadows (e.g. Jones *et al.,* 1995; Holzel and Otte, 2003; Trueman and Millett, 2003; Donath *et al.*, 2007; Edwards *et al.*, 2007; Kiehl and Pfadenhauer, 2007; Klimkowska *et al.,* 2007; Stevens and Wilson, 2012; Cornish and Hooley, 2012; Starr-Keddle and Barrett, 2012). This technique involves choosing a suitable species-rich meadow to act as a donor site, cutting and baling the green hay, when the maximum number of species have set seed, and immediately taking the material to the pre-prepared receiver site and strewing the hay across the field. This method has been found to create a plant community similar to that of the donor site (Jones *et al.,* 1995; Trueman and Millet, 2003; Edwards *et al.*, 2007; Kiehl *et al.,* 2010; Cornish and Hooley, 2012; Starr-Keddle and Barrett, 2012) and also inherently means that the provenance of the material is known. The use of local provenance seed is important, as discussed previously. It should also mean that the seeds will be fresh and at maximum viability compared to 'commercial' seed whose storage history, and therefore viability, will be unknown to the creation project manager. Other advantages of hay strewing include: the transfer of insects (Kiehl and Wagner, 2006), the transfer of mycorrhizae (Trueman and Millett, 2003), the transfer of mosses and lichens (Stroh *et al.*, 2002; Poschlod and Biewer, 2005), the inclusion of rare species (Kiehl *et al.,* 2010) and the successful establishment of the more difficult species including orchids (Trueman and Millett, 2003). Additionally, the material can provide protection from high temperatures, the magnitude of temperature changes and from drying out by acting as a mulch, aiding germination and protecting the new seedlings (Eckstein and Donath, 2005; Donath *et al*., 2006) and the method involves non-specialized farm machinery and is therefore cheaper than methods such as brush harvesting (Kiehl *et al.,* 2010; Vander Mijnsbrugge *et al*., 2010). In this thesis, green hay was used in preference to dried hay and 'green hay' is what is referred to by 'hay' throughout.

A disadvantage of green hay strewing may be a lack of knowledge of the exact composition of the seed present within the hay, meaning that it is difficult to assess success, although surveys prior to harvesting improve this situation (Kiehl *et al.,* 2006; Hedberg and Kotowski, 2010). However, Hedberg and Kotowski (2010) stated in their review of the literature that, when hay strewing is used, a survey of the donor field is not always carried out, making an assessment of the success of the transfer of the community impossible. In addition to surveying the donor meadow, Kiehl *et al.* (2006) grew a sample of the hay in a greenhouse to assess the composition and viability of seed within it and recorded the phenological status of the plants at harvest, to increase their knowledge of the composition of the hay and therefore the potential species that could be transferred. They used these data, plus a list of species that were not found in the hay, but were present in the donor meadows[1] and also the new species on the receiver site, to create a list of potentially transferable species (Kiehl *et al*., 2006). Of 220 species found on the donor sites, 92 were found to be potentially transferable (Kiehl *et al*., 2006).

Other potential disadvantages associated with this method include the wide range of seed set dates amongst meadow plants and also seed size, causing its loss from hay in transit (Trueman and Millett, 2003). The range of seed set dates means that hay may need to be harvested at different times of the year (Trueman and Millett, 2003; Walker *et al.,* 2004). Potential, practical complications include the availability of hay from suitable species-rich source sites (due to their paucity), the distance to the nearest source site, the difficulties involved in calculating the costs for these projects (e.g. for grant applications) and the complexity in planning and organising the implementation of hay strewing due to uncertainty of cut dates (and in years when the weather is very wet, complete unavailability of hay). The hay also needs to be cut, baled and strewn all on the same day to prevent the bales heating up and killing the seed (Trueman and Millett, 2003).

Another consideration is the potentially negative effect of harvesting green hay on the source meadow, due to the removal of a high proportion of the seeds compared to the usual hay-making process. This involves the hay being cut, turned and dried before it is removed, hence allowing the seeds to

[1] Including individual species such as *Carex caryophyllea* that were not ripe or had already released their seed at the time of harvesting.

fall from the hay onto the meadow (Stevenson *et al.,* 1997; Vander Mijnsbrugge, 2010). This has led to recommendations to use source meadows in rotation (Stevenson *et al.,* 1997; Trueman and Millett, 2003; Vander Mijnsbrugge, 2010) or to only cut a proportion of the meadow for green hay each year (Trueman and Millett, 2003; Natural England, 2010), although there does not appear to be any published research on the effect of harvesting green hay on donor meadows. Gamble (2015) reports that the Hay Time project has been following Natural England's recommendations (only cutting a third of the meadow and not repeating harvesting for at least three years) and that monitoring of the donor meadows indicates no adverse effect of the green hay collection.

### 1.8 Establishment success

Despite the overall success of created and restored species-rich grassland communities, a number of characteristic grassland species have been found to establish and/or persist poorly in these re-created communities (Pywell *et al.* 2003; Hewins *et al.,* 2012). These grasslands tend to lack some of the characteristic species of species-rich NVC communities (Walker *et al.,* 2004) – i.e. habitat specialists that tend to be stress-tolerators and species of infertile soils; e.g. *Succisa pratensis* and *Silaum silaus*, both MG5 *Cynosurus cristatus-Centaurea nigra* community constancy I species[2] (Pywell *et al.,* 2003; Hewins *et al.,* 2012). Stevens and Wilson (2012) reported upon a survey of agri-environment scheme funded grassland creation projects on a

[2] The NVC collects percentage frequencies of recorded species into constancy classes, as follows: class V, frequency range 81-100%; IV, 61-80%; III, 41-60%; II, 21-40%; I, 1-20% (Rodwell *et al.*, 1991 *et. seq.*).

range of grassland types, including MG5. They stated that there were often large differences in the vegetation when compared with semi-natural communities, although it seemed clear that these schemes could result in meadows that could be identified as UK BAP priority habitats.

A review of the literature gives a list of species which 'on average' perform poorly (Table 1.1), although it should be noted that many studies do not report on the success (or otherwise) of many species, or are only introducing a limited number of species, which may artificially inflate or deflate the figures calculated for this table, e.g. *Dactylis glomerata* may not be expected to be poor-performing and it may have been expected to find orchid species' in the table (i.e. described as poor-performing). Neither *D. glomerata* nor orchids are generally included in seed experiments, however, the small number of (hay strewing) experiments found that reported upon this species had a lack of success with *D. glomerata* and relatively high success with orchids, therefore inflating the poor success value of *D. glomerata* and deflating the poor success rate of orchids. It should be noted that Table 1.1 is not meant as an exclusive list of all species that are poor-performing, but is only a list of those species it was possible to gain enough information on from a review of the literature.

Table 1.1: Poorly performing* MG5 *Cynosurus cristatus-Centaurea nigra* species, with their NVC constancy classes (Rodwell, 1992), taken from a review of the literature**

| **Species and MG5 Constancy** | | **Establishment success (S=successful; PE=Poor establishment; DNE=Did not establish)*** ** | **Articles with information about transfer/establishment** |
|---|---|---|---|
| *Lotus corniculatus* | V | S-20%; PE-40% | *a, b, f, h, i* |
| *Dactylis glomerata* | IV | PE-50%; S-33% | *h, i* |
| *Trifolium pratense* | IV | PE-44% | *b, f, h, i* |
| *Luzula campestris* | III | PE-33%; DNE-33%; S-16% | *h, i* |
| *Ranunculus acris* | III | PE-28% | *i* |
| *Rumex acetosa* | III | PE-57%; S-28% | *i* |
| *Arrhenatherum elatius* | II | DNE-66% | *i* |
| *Briza media* | II | DNE-50% | *c, f, g, i* |
| *Galium verum* | II | DNE-66% | *i* |
| *Heracleum sphondylium* | II | DNE-50% | *h, i* |
| *Lathyrus pratensis* | II | DNE-43% | *i* |
| *Poa trivialis* | II | S-33% | *i* |
| *Veronica chamaedrys* | II | DNE-43% | *i* |
| *Agrimonia eupatoria* | I | DNE-66%; PE-33% | *a* |
| *Alchemilla* sp. (3spp.) | I | DNE-100% | *i* |
| *Avenula pubescens* | I | DNE-100% | *i* |
| *Betonica officinalis* | I | DNE-71.43%; PE-14.29% | *i* |
| *Cardamine pratense* | I | DNE-66%; PE-33% | *i* |
| *Carex caryophyllea* | I | DNE-100% | *i* |
| *Carex flacca* | I | DNE-60%; PE-40% | *f, i* |
| *Carex panicea* | I | PE-100% | |
| *Colchicum autumnale* | I | DNE-100% | |
| *Conopodium majus* | I | DNE-83.33% | *h, i* |
| *Festuca ovina* | I | DNE-100% | |
| *Filipendula ulmaria* | I | DNE-33.33%; PE-66.67% | *i* |
| *Juncus articulatus* | I | PE-100% | *i* |
| *Knautia arvensis* | I | PE-50% | *d, g, i* |
| *Ophioglossum vulgatum* | I | DNE-100% | *i* |
| *Potentilla erecta* | I | DNE-57.14%; PE-14.29% | *i* |
| *Potentilla reptans* | I | DNE-50%; PE-50% | *i* |
| *Silaum silaus* | I | DNE-33.33%; PE-66.67% | *a, g* |
| *Succisa pratensis* | I | DNE-57.14% | *e, f, g, i* |
| *Vicia cracca* | I | DNE-57.14% | *i* |

*Species included are those with a low percentage success rate or high PE or DNE success rates or a combination of low success and high PE or DNE.
**Includes a variety of project starting points (creation, restoration etc.) and a variety of introduction methods.
***Establishment success calculated as a percentage using studies where these species were mentioned (references in third column of table).
[a]Hopkins *et al.,* 1999; [b]Hofmann and Isselstein, 2005 (seed mix incl UK sp); [c]Jongepierova *et al.*, 2007 (Festuco-Brometea target); [d]Nordbakken *et al.,* 2010 (seed mix 5/6 UK sp); [e]Oster *et al.,* 2009 (seed mix 13/16 UK sp); [f]Pakeman *et al.,* 2002; [g]Pywell *et al.,* 2003; [h]Rayner, 2005; [i]Trueman and Millett, 2003.

To increase the similarity of created species-rich grasslands to 'target' semi-natural communities, it is important to find ways to increase the success of these poorly performing and missing species (Pakeman *et al.,* 2002; Smith *et al.,* 2003), both in initial creation attempts and in introductions and re-introductions into already established created grasslands (i.e. 'enhancement' or 'phased introduction' projects). For many of these poorly performing species, little is known about the necessary conditions for establishment and persistence or how these might be provided (Wagner *et al*., 2012).

There are several possible reasons why species may not be present in a created meadow (Figure 1.3). It should also be noted that species that have been recorded as missing from a meadow may not have been recorded in the survey, but may actually have been present. This may be because the species is present only in small numbers or only in one or two patches i.e. the species has established and is persisting, but is not spreading, as with some of the species studied by Pakeman *et al.* (2002). The period of time since creation is also an important factor associated with the spread of species: created species-rich meadows are known to need time to develop the complexity and evenness of species abundance associated with their semi-natural target habitats (McDonald, 2001; Willems, 2001; Walker *et al.,* 2004; Rayner, 2005; Woodcock *et al.,* 2006). It is likely that the number of species in created meadows increases over time, subject to appropriate management etc., although more research is required (Stevens and Wilson, 2012; Hewins *et al*., 2012). In their survey of created and restored meadows, Stevens and Wilson (2012) found that the most species-rich sites had been established for over 10 years. Another consideration regarding the absence of species is that, where populations are small, a chance event can lead to the loss of the species from the created meadow.

The length of time since the creation of the meadow in the definition of 'missing' from a created meadow is also important for other reasons. Some species can take a number of years to become established e.g. orchid species can take several years to appear (Trueman and Millett, 2003) and species such as *Succisa pratensis* can take four years to flower and hence become more visible (Adams, 1955). This means that monitoring needs to continue for a number of years to truly establish whether a species is missing or just not yet established from the introduction. Conversely, it may also be that the species did establish from the original introduction, but was then lost – i.e. the species is missing due to the management on the site, not the introduction methodology.

The abiotic and biotic conditions, mentioned in Figure 1.3, that can affect the performance of a species include: the soil conditions (e.g. soil fertility, pH, hydrology and the soil biota), the management regime, the traits of the individual plant species, the effect of competition amongst the sown species, with weed species and/or with any existing vegetation, and the stage of succession reached when aiming to improve the diversity in an existing sward. Also relevant when introducing new species into an existing

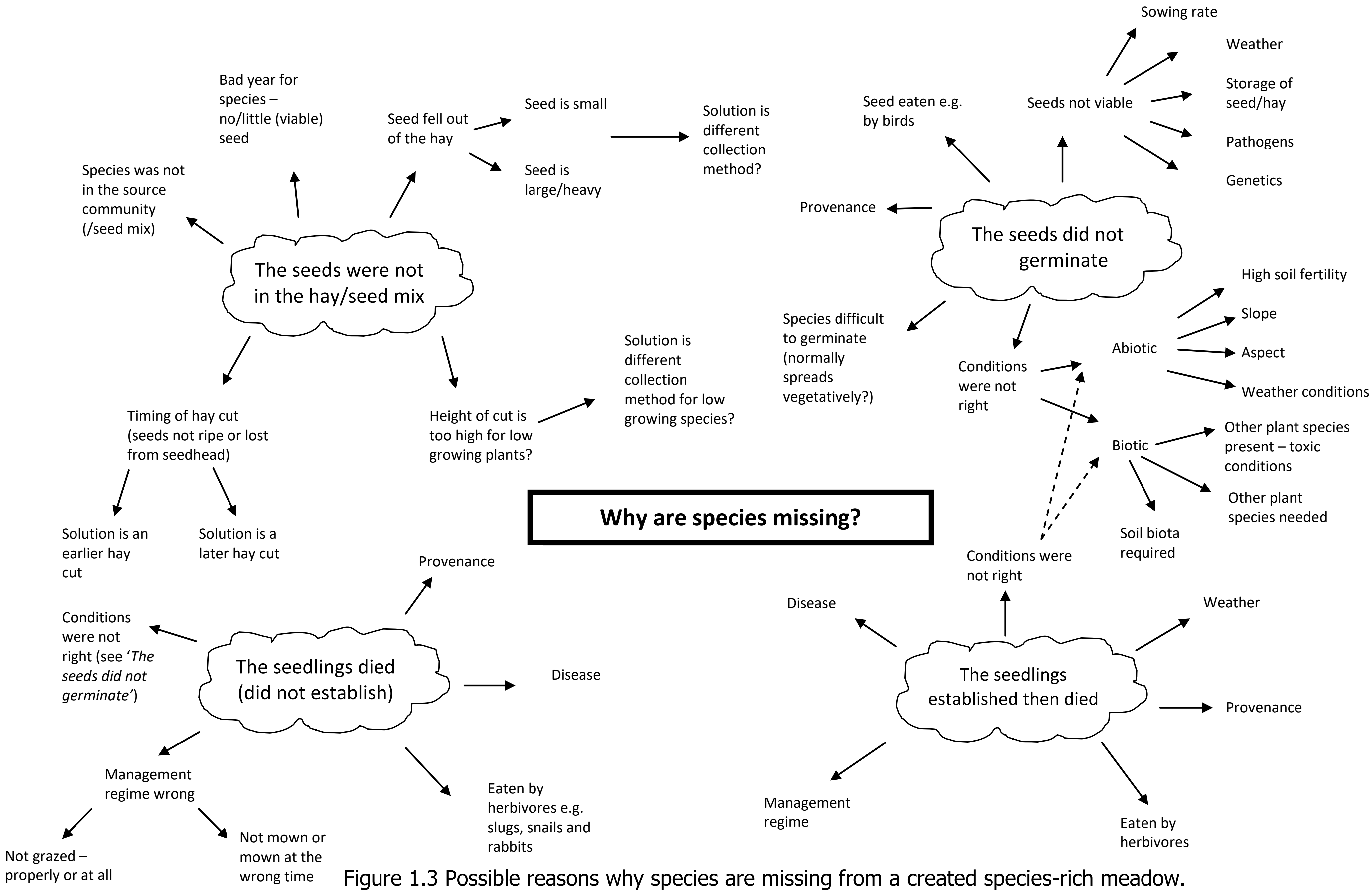


Figure 1.3 Possible reasons why species are missing from a created species-rich meadow.
References include: Grime *et al.* 1988; Edwards and Crawley, 1999; Jones and Hayes 1999; Smith *et al.,* 2000; Bullock *et al.*, 2001; McCrea *et al.,* 2001; Ehrlen *et al.* 2006; Beltman *et al.*, 2007; Jongepierova *et al.,* 2007; Scotton *et al.,* 2009; Allen *et al.*, 2010. For more detail, see text.

community, as may be the case when introducing or re-introducing missing species into a meadow, are: the 'invasibility' of the community (Burke and Grime, 1996; Wilson *et al.,* 1996; Franzen, 2001; Foster *et al.,* 2002; Kleijn, 2003; van Ruijven, 2005; Critchley *et al.,* 2006) and the potential need to create gaps in the vegetation, by a chosen method, to allow the establishment of the introduced species (e.g. Bullock *et al.,* 2001; Cousins and Lindborg, 2008; Fleischer *et al.,* 2013).

In summary, the main issues that can affect the establishment of species introduced into a new or existing community are:

- the effect of the management regime
- the effect of plant traits/species' specific characteristics
- the effect of the site conditions
- the invasibility of the established community and competition
- succession and the conditions present in the meadow.

There are a number of possible ways to improve the performance of the unsuccessful species in creation and restoration attempts. These include: the use of plug plants or turf (where appropriate) for specific species; the phased introduction of species over one season through the phased cutting of the source meadow when using green hay and by the phased introduction of species over different years by introducing missing species into the established grassland. When strewing hay, the seeds for the phased introduction could be introduced by transferring all of the hay left standing for the second cut or by selectively harvesting the seed of individual species (Edwards *et al.*, 2007); for example, by vacuum sampling or hand collecting (Stevenson *et al.*, 1997; Riley *et al.,* 2004). These introductions would ideally be implemented with the appropriate ground preparation and aftercare/management to give the species the best chance of establishing and persisting.

A number of studies suggest the phased sowing of seed to introduce missing species (e.g. Pakeman *et al.,* 2002; Pywell *et al.,* 2002; Smith *et al.,* 2002; Pywell *et al.,* 2003; Smith *et al.,* 2003; Walker *et al.,* 2004; Edwards *et al.,* 2007; Jongepierova *et al.,* 2007; Smith *et al.,* 2008), although, as yet, there is little evidence to support this idea. Studies suggest that particular species are not ecologically adapted to either establish, persist or spread in the initial sowing (Pakeman *et al.,* 2002; Pywell *et al.,* 2003; Walker *et al.,* 2004; Jongepierova *et al.,* 2007). This implies that these species should be introduced when the vegetation has developed sufficiently; when management has produced suitable conditions (Pakeman *et al.*, 2002) and environmental conditions are more favourable and less dynamic (Walker *et al.,* 2004). Evidence for a positive effect of facilitation (by 'colonizer' or 'facilitator' species for or on other species) has yet to be reported (e.g. Dunn and Tallowin, 2012 and Martorell *et al.*, 2015).

Dunn and Tallowin (2012) suggest that the terminology used in ecological discussions of creation projects needs to be refined, as the process involved is more accurately the reverse of the normal succession process (bare ground to meadow to woodland/nutrient poor to nutrient rich) and as creation sites are often more fertile than the target habitat. Therefore, species that are known as early successional species within the creation literature would be better described as early stage restoration species. This practise will be followed in this thesis.

The creation of gaps in the sward is known to be important to allow the recruitment of seedlings to the community (Smith *et al.,* 2000; Bischoff, 2000; Turnbull *et al.,* 2000; Bullock *et al.,* 2001). As is grazing at the correct time and neither too heavy or too light (Bullock *et al.,* 1995; Bullock *et al.,* 2001; Wagner *et al.,* 2012; Cornish and Hooley, 2012) or some other method of removing the vegetation and creating disturbance (Trueman and Millett, 2003; Hofmann and Isselstein, 2004; Edwards *et al.,* 2007). This is explored further in Chapter 5.

Walker *et al.* (2004) suggested that more information is needed on the functioning of species-rich grasslands to inform techniques with the objective of improving the performance of species that tend not to establish. This includes information regarding the soil microbe, fungal and faunal communities, for example, studies on their functioning, their assembly during species-rich grassland creation and the role of soil microbes and fungi during succession. Walker *et al.* (2004) also suggested the need for more information regarding the importance of food webs during grassland succession and the development of trophic and pollination relationships. This may be reliant on the presence of plant species that currently perform poorly in grassland creation schemes and/or may be dependent on the arrival of invertebrate species into the new grassland site, either by natural dispersal or by introduction (Walker *et al.,* 2004). As previously mentioned, invertebrates will be introduced along with the hay when sites are created by hay strewing (Kiehl and Wagner, 2006).

As mentioned, certain species may be more difficult to encourage to germinate and establish successfully from seed. This includes orchids, a number of species of which are associated with hay meadows (Rodwell, 1992). *Ex situ* propagation, with later introduction into meadow sites is one possible solution (McKendrick, 1995; Scade *et al*., 2006; Ashmore *et al*., 2011; Krupnick *et al*., 2013) and is the focus of Chapter 6 for one orchid species, in this thesis. However, even if other methods of introduction are used, there is still a need to ensure that environmental conditions are correct for the species to persist and that management is in place to help the species spread from the localized introduction sites (Wells*,* 1989; Bischoff, 2002).

Pywell *et al.* (2003) analysed the results of 25 grassland creation and restoration experiments. They found that generalist species performed well, whereas other species, particularly those that are specialists of this habitat type, perform poorly. Hewins *et al.* (2012) in a review of 36 sites where species-rich grassland creation had been carried out under option HK8[3] of the Higher Level Stewardship Scheme[4] found that, although 17% of the sites could be described as good quality BAP habitat and 47% met lower definitions of BAP grassland, positive indicator species, particularly high value ones, were still lacking. It is crucial to find ways to improve the success of poorly performing species for a number of reasons, including to increase the similarity of created species-rich grasslands to 'target' semi-natural communities (Pakeman *et al.,* 2002; Smith *et al.,* 2003) and because these species may be key to food and pollinator webs in grasslands, for example, being food plants of invertebrates that are important for conservation (Pywell *et al.,* 2003).

In summary, it is known that species-rich grassland creation is possible and that green hay strewing is a successful grassland creation technique that results in a community similar to that of the donor site, includes rare species and also species other than plants (e.g. orchid mycorrhizae), although the establishment of some species appears to be less likely. The University of Wolverhampton has a history of research into habitat creation (e.g. Jones, 1993; Besenyei, 2000; McCrea *et al.,* 2001; Trueman and Millett, 2003; Rayner, 2005), including the development of the species-rich meadow creation technique of green hay strewing. The current project investigates methods of increasing the numbers of appropriate species in created meadows, both at the initial creation and when the created meadow is

[3] HK8 is the option under which species-rich grassland creation is funded.
[4] After five years of the scheme.

established (i.e. enhancement), with a focus on meadows classified as MG5 *Cynosurus cristatus-Centaurea nigra* hay meadows within the British National Vegetation Classification (NVC) system (Rodwell, 1992) and also to increase the amounts of meadow species that are already established in created meadows but at low frequency or abundance to increase the evenness of the meadow and to reduce the risk of these species being lost from the site. The target species for each individual experiment vary, because some created meadows will have some species missing that are normally 'easy' to establish and also may have species that are normally 'difficult'. In addition, source meadows will not contain all the 'difficult' target species.

In short, the rationale for the project is to improve the established green-hay strewing technique by investigating methods to increase the success rate with species that do not easily transfer.

## 1.9 Aims and Objectives

The thesis seeks to achieve the following aims:

To increase the number of different species that establish in created species-rich meadows, using sequential green hay strewing.

To increase the species-richness and evenness of species that establish in created species-rich meadows, using sequential green hay strewing.

To assess techniques and media for axenic cultivation of an orchid species (*Dactylorhiza fuchsii)* associated with this habitat type.

Objectives:

To undertake multiple (phased) strewings of green hay over a number of years, from a species-rich donor meadow for the creation of a new species-rich meadow.

To introduce additional species into an existing created species-rich meadow using green hay strewing; i.e. enhancement of an existing created meadow (phased sowing).

To compare the effect of different levels of disturbance on the success of introducing species into an existing species-rich meadow using green hay strewing; i.e. enhancement of an existing created meadow.

To investigate the effect of grazing on the success of introducing species into an existing created species-rich meadow using green hay strewing; i.e. enhancement of an existing created meadow.

To compare symbiotic and asymbiotic media, with and without additions of fungi and nutrients, using axenic seed germination and propagation techniques, on the meadow orchid species *Dactylorhiza fuchsii*.

# Chapter 2

# Materials and Methods[5]

## 2.1 Site selection

Farmers/landowners in the Midlands who were members of the 'Higher Level Stewardship Scheme' were sent a questionnaire[6] through the Midlands Land Management Office of Natural England in January 2011. The questionnaire identified farmers who were interested in being involved in this research and gathered information to help identify which of these may have suitable sites. A number of potential sites were shortlisted and then visited. Sites were assessed for the suitability of the fields (size (i.e. not too small for the layout of a replicated experiment), little/no slope, vegetation that suggested suitable soil conditions) and availability of source meadow hay, grazing animals and suitable machinery. Sites were also chosen for their suitability for the NVC MG5 *Cynosurus cristatus-Centaurea nigra* community (Rodwell, 1992), as described in Section 1.2. They were selected, for example, by assessing the existing vegetation, the altitude/location of the site and also their susceptibility to flooding. Using these criteria, two sites in Herefordshire were chosen: these were Cae Gross at Lower Turnant (Chapter 4) and Golden Field at The Bryn (Chapter 5).

The creation of the Birmingham and the Black Country Nature Improvement Area (NIA) (Wildlife Trust for Birmingham and the Black Country, 2012)

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[5] Due to the very different techniques used, the materials and methods for the chapter on axenic seed germination of *Dactylorhiza fuchsii (*Chapter 6) are described in that chapter, not here.
[6] Approval for the questionnaire was given by the University of Wolverhampton Faculty of Science and Engineering Life Sciences Ethics Committee.

provided the opportunity for more research sites. In 2012 and 2013, a number of potential NIA sites were visited and Castle Vale (Chapter 3) was chosen owing to the suitability of the field, using the criteria above[7].

## 2.2 Vegetation surveys

The vegetation was surveyed at all of the source and receiver sites before the green hay was collected from the source meadow and before any treatment was applied to the receiver meadow. The details of the timing of surveys and numbers of quadrats surveyed are given in the individual experiment chapters. The author undertook all surveys (to/which reduced observer variability). Vegetation was surveyed using 1 m x 1 m quadrats, located using random co-ordinates and recording estimates of percentage cover by eye of all vascular species rooted within each quadrat. Percentage cover of bare ground was also estimated. The quadrat size was chosen after a review of the literature showed that no standard size is used in grassland surveys (e.g. Rodwell, 1992; Stevenson *et al.*, 1997; Pywell *et al.,* 2002; Gilbert *et al.*, 2003). The grassland surveys for the NVC assessments used 2 m x 2 m quadrats (Rodwell, 1992). However, 1 m x 1 m quadrats are quicker to assess than 2 m x 2 m ones, meaning that more quadrats can be surveyed within a given time period and they can also be surveyed from outside the quadrat, without trampling the vegetation to access the centre. Previous work on created meadows at the University of Wolverhampton also

[7] A number of other sites were experimented upon, in Herefordshire and in the NIA, but have not been included in this thesis owing to word count constraints.

used 1 m x 1 m quadrats e.g. Jones *et al*. (1995), Besenyei (2000) and Rayner (2005), therefore, the same size was chosen to enable comparison with these earlier studies. Where vegetation could not be identified to species level, it was identified as far as possible at the time and recorded as such (e.g. *Dactylorhiza* sp.). Nomenclature follows Stace (2011) and a full list of species is provided in Appendix 2.1, together with the abbreviations used within the thesis results.

## 2.3 Receiver meadow pre-treatment

The vegetation in the receiver meadows was pre-treated in a number of ways, depending on the type of experiment and the content of the existing vegetation (Table 2.1). All sites were cut and arisings[8] removed, then: Castle Vale, an experiment to investigate the effect of introducing hay in consecutive years, was pre-treated with glyphosate to kill off the existing sward, two weeks before strewing, because the existing vegetation was species-poor and dense, after years of no management. Previous work suggested glyphosate for the best results in this situation (Trueman and Millett, 2003) and this was the approach of the funder. Cae Gross, an experiment to investigate if missing species can be introduced into existing species-rich communities, was pre-treated uniformly with a power harrow, to create gaps in the sward needed for the establishment of new seedlings (Bullock *et al.,* 2001). The machinery was set to create disturbance in the thatch, but minimal disturbance of the soil, i.e. to a maximum depth of about

[8] i.e. all the cuttings and also potentially, thatch/moss from the bottom of the vegetation, depending on the machinery used.

1 cm. The vegetation cover was approximately 50% after the disturbance had been applied. Golden Field, used for an experiment investigating the effects of different levels of disturbance, was pre-treated with no-disturbance, low-disturbance (three passes of the machine) and high-disturbance (six passes of the machine) using a power harrow. The machinery was again set to create disturbance in the thatch, but minimal disturbance of the soil, i.e. to a maximum depth of about 1 cm. The vegetation cover was approximately 75% after the low disturbance treatment and 50% after the high disturbance treatment.

Herbicide was used at Castle Vale because the existing vegetation was not desirable and herbicide has been shown to be an effective technique when carrying out meadow creation where the vegetation is not desirable (Jones *et al.,* 1995; Besenyei, 2000). Castle Vale had a second strewing of hay in the following year. Unfortunately, circumstances meant that this occurred on top of the existing vegetation before it was cut. Ideally, the meadow would have been cut and removed before the second strewing took place.

The aim of the disturbance on Golden Field and Cae Gross was to create gaps within the existing sward to allow establishment of the introduced seed without killing off the existing vegetation (Figure 2.1). Disturbance was used instead of herbicide on these sites, because the aim was to introduce species into existing meadows, although the sites had a range of initial species-richness. See Appendix 2.2 for photographs of the machinery used.

Table 2.1: Pre-treatment of vegetation in receiver meadows

| Chapter number | Receiver | Source | Experiment | Pre-haying treatment (after cut and remove operation) | Year(s) hayed |
|---|---|---|---|---|---|
| **3** | Castle Vale | Eades | 2 strews from the same source in consecutive years | Glyphosate treatment to kill off the existing sward. Dead material was not removed. | 2013 and 2014 |
| **4** | Cae Gross | Pikes Farm | Introducing new species to existing species-rich vegetation | Disturbance with power harrow, equal to low level disturbance as above. *Effect:* removal of existing vegetation and some surface disturbance of the soil (1-2 cm). | 2011 |
| **5** | Golden Field | Three Yew Trees | Introducing new species to existing species-rich vegetation and the effect of disturbance (and grazing) | No disturbance, low disturbance, high disturbance (power harrow). *Effect:* as Cae Gross | 2011 |

(a) Low disturbance (mobile phone for scale).

(b) High disturbance (mobile phone for scale).

(c) Low disturbance.

(d) High disturbance.

Figure 2.1 (a-d): Disturbance produced by the power harrow at Golden Field.

## 2.4 Green hay collection and strewing

For all sites, the source meadow was cut (at a height of 2-3 cm), baled, transported to the receiver meadow and green hay strewn at the receiver on the same day, the detail of the timing differs between sites and so is given in the individual chapters. The size and shape of the bales differed between the sites, due to the equipment available (Appendix 2.2) and the difficult access to some of the source meadows (Table 2.2). The application rate of the hay was 1:3 throughout, based on previous experiments (e.g. Besenyei, 2000; Rayner, 2005).

The detail of the experimental layouts differed between sites and so is given in the individual experimental chapters.

Table 2.2: Methods used for baling and strewing for each receiver meadow

<table>
<tr><th>Receiver</th><th>Source</th><th>Size of bales</th><th>How strewn</th></tr>
<tr><td>Castle Vale</td><td>Eades</td><td>Large round bales: 90 cm (36”) x 45 cm (18”) (Figure 2.2). Baler attached to tractor</td><td>Bales placed across the meadow (by a tractor) and then the hay was spread by hand</td></tr>
<tr><td>Cae Gross</td><td>Pikes Farm</td><td rowspan="2">Small round bales: 52 cm (20") x 55 cm (22"). Baler attached to Bank Commander</td><td rowspan="2">Bale placed across the meadow from a trailer, rolled out and then spread using a tedder</td></tr>
<tr><td>Golden Field</td><td>Three Yew Trees</td></tr>
</table>

Figure 2.2: Large bales as used at Castle Vale.

## 2.5 Data handling and analysis

### 2.5.1 Summary statistics

A number of summary statistics were calculated in order to allow analysis and comparison of the data. These were as follows:

#### 2.5.1.1 Total number of species per site and species-richness for each quadrat

The total number of species found at each site and species-richness for each quadrat (i.e. number of species present in each 1 $m^2$ quadrat) were used to describe the species-richness of the sites and to compare the sites and years. They were then used to compare the species present, for example, how many of these species are desirable: i.e. slow growing species typically found in meadows, cf. faster growing species more likely to become dominant or species more typically found in other habitats (e.g. *Rumex obtusifolius*, tree seedlings). Some of these less desirable species could include those of the MG5 community, e.g. *Arrhenatherum elatius* and *Dactylis glomerata*. See section 2.5.1.2 for explanation of definitions of desirability as used in this thesis. The species and their characteristics are described in the results and discussion sections of the relevant chapters.

The number of species for each quadrat was then used to calculate the mean number of species present in each quadrat for each site. This calculation was also used to compare the sites to each other, as with the total number of species.

### 2.5.1.2 Species desirability or undesirability

Species were also classified as desirable, neutral or undesirable in an MG5 *Cynosurus cristatus-Centaurea nigra* community (Rodwell, 1992) using the method shown in Table 2.3. Species were initially classified as desirable, neutral or undesirable using the CSR strategy model (Grime, 1974, 1977, 1979) for each species; e.g. ruderals (R) were classified as neutral, as the hay meadow management would mean that they would not persist (Trueman and Millett, 2003). However, this led to some species being classified incorrectly; for example, *Crataegus monogyna* (a SC strategist) is desirable under this categorization (Table 2.3). Therefore, the initial classification was altered by first taking into account the type of species – i.e. tree species were moved into undesirable.

The next step was for grassland species that were non-MG5 community species. For these species, the associated floristic diversity score from Grime *et al.* (1988) was taken into account. For example, species with a score of five were classified as desirable. *Carex nigra* has a floristic diversity score of two, but Grime *et al.* (1988) states that although this species has a low associated floristic diversity on acid soils, it often has a high associated floristic diversity on soils with a high pH. This species is also a S/SC strategist, which means it would fall into the desirable category if it was on the MG5 community species list. The combination of these factors meant this species was moved from the undesirable category (from its diversity score) to neutral.

The last step was for MG5 species: these were left with their original classification as desirable, neutral or undesirable from their CSR strategy except for *Heracleum sphondylium* (CR strategist) and *Juncus* spp. *Heracleum sphondylium* was moved from undesirable to desirable as, from the researchers' experience, it normally does not behave aggressively in annually cut meadows. *Juncus* spp. were moved from desirable to neutral, as they can become dominant in some meadows, e.g. in damp meadows or wet years.

Table 2.3: Method used for classifying species as desirable, neutral (neither desirable nor undesirable) and undesirable for hay meadows, see explanation in text

<table>
<tr><td colspan="3">First classify species using their established (CSR) strategy as in Grime (1974, 1977, 1979)</td></tr>
<tr><th>Desirable</th><th>Neutral</th><th>Undesirable</th></tr>
<tr><td>SR/ with…<br>SR<br>SC/ with...<br>SC<br>S<br>S/<br>R/ with S<br>CSR<br>CS/…<br>CR/ with S</td><td>R<br>C/with S</td><td>No S<br>C</td></tr>
<tr><td colspan="3">THEN alter above classification for individual species as follows:</td></tr>
<tr><td></td><td></td><td>Woodland/hedgerow/scrub species</td></tr>
<tr><td>Grassland species, non-MG5 – with an associated floristic diversity score (from Grime, 1988) of 5</td><td rowspan="2">Grassland species, non-MG5 – with an associated floristic diversity score of 3 or 4</td><td rowspan="2">Grassland species, non-MG5 – with an associated floristic diversity score of 1 or 2. Except Carex nigra (moved from U to N due to combination of its CSR strategy (S/SC) and its higher diversity score on higher pH soils)</td></tr>
<tr><td>MG5 grassland orchid species</td></tr>
<tr><td colspan="3">MG5 species stay as their CSR D/N/U except:</td></tr>
<tr><td>Heracleum sphondylium moves from U to D as, from researchers’ experience, it normally does not behave aggressively in annually cut meadows (CR strategist)</td><td>Juncus species’ move from D to N, as they can become dominant in some meadows e.g. in damp meadows or wet years</td><td></td></tr>
</table>

### 2.5.1.3 Species diversity measures

Species diversity measures are used in the analysis of abundance data and can be used to compare similar sets of data. Magurran (2004) recommends the use of the following three measures: Simpson's Index, Simpson's Measure of Evenness and the Berger-Parker Index, as they do not assume any underlying species abundance distribution, so these are used in this research. Evenness describes the differences in species' abundances in a community; i.e. a community in which all species have similar numbers of individuals would be rated as exceptionally even. Diversity indices such as Simpson's Index, combine information on species richness and evenness and is, therefore, not a true measure of evenness. Magurran (2004), therefore, recommends the use of Simpson's Measure of Evenness, in addition to Simpson's Index. The Berger-Parker Index measures dominance (the corollary of evenness).

(i) Simpson's Index (D)

The formula for Simpson's Index, for use with a finite community, is shown below.

$$D = \sum \left( \frac{n_i [n_i - 1]}{N [N-1]} \right)$$

$n_i$ = the percentage cover in the $i^{th}$ species
N = the total percentage cover
(Magurran, 2004)

Diversity decreases as Simpson's Index increases, therefore, the complement (1-D), or the reciprocal (1/D), is usually used. However, this study used Kemp's transformation (-lnD), as suggested by Magurran (2004) to avoid problems with the former methods. Kemp's transformation of Simpson's Index has the values 0-1 and increases as diversity increases.

(ii) Simpson's Measure of Evenness ($E_{1/D}$)

$$E_{1/D} = \frac{(1/D)}{S}$$

S = the number of species in a sample

$E_{1/D}$ has values between 0 and 1 and increases as evenness increases

Simpson's Measure of Evenness increases as evenness increases (i.e. it increases as the abundances of all the species present become more similar).

(iii) The Berger-Parker Index (d)

This index describes the proportional abundance of the most abundant species and, hence, is a measure of dominance in a community (i.e. the degree to which one or a few species dominate a community; Magurran, 2004). It increases as the abundance (i.e. dominance) of the most abundant species increases.

$$d = N_{max} / N$$

where $N_{max}$ = the percentage cover of the most abundant species and N is the total percentage cover

d has values between 0 and 1

(iv) The Czekanowski coefficient ($C_s$)

This index is a similarity measure and is also known by several other names, including Sorenson's measure and the Bray-Curtis coefficient. The disadvantage of this measure is that it does not take into account the relative abundance of species and such measures also depend on the sites being exhaustively surveyed – an aim that is difficult to achieve (Magurran, 2004).

| | |
|---|---|
| $C_s = 2a/(2a+b+c)$ | Where a = the total number of species present in both sites, b = the number of species present only in site 1 and c= the number of species present only in site 2<br><br>$C_s$ has values between 0 and 1 |

### 2.5.2 Percentage frequency tables

For each species found in quadrats in each meadow and year, the percentage of quadrats that the species was recorded in (i.e. its percentage frequency) was calculated and then displayed in a percentage frequency table to enable comparison between the years (at the receiver meadow) and the source meadow.

### 2.5.3 Statistical tests

Parametric statistical analyses were performed on SPSS Statistics for Windows Version 20.0 (IBM, 2011). ANOVA tests were run on normalized data ($\log_{10}$ transformed or square root as appropriate) and homoscedasticity was checked with Levene's test. Where data could not be transformed to normality, non-parametric equivalents were used instead. Where data were recorded from the same plot on more than one occasion, repeated measures ANOVA was used, on the mean data per plot; where sphericity could not be assumed (Mauchly's Test), the Huynh-Feldt output was interpreted where $\varepsilon > 0.75$ (following Laerd Statistics 2013).

The source and the baseline were not included in this analysis, the latter because it had not been possible to identify the treatment blocks in advance of the strewing.

### 2.5.4 Ecological multivariate analyses

Multivariate analysis is also used to examine complex data sets in ecology, particularly where a number of variables are related to each other (Legendre and Legendre, 1998). There are several techniques available, two of which are used in this study: ordination and classification.

#### 2.5.4.1 Ordination

Ordination was carried out using the computer package Canoco 5 (ter Braak and Smilauer, 2012) and it was also used to create the scatter diagrams/ordination plots in this thesis. Where PCA was the more appropriate technique, data were analysed on a correlation matrix. Scaling focusing on correlation between response variables was used[9]. Response scores were divided by their standard deviation. The resulting graphs were centred by species. This means that when interpreting ordination diagrams

[9] In PCA (and DCA), when a biplot of the species and sample data is produced, the data from one plot is superimposed on the other so that, in effect, each axis has two scales – one for the sample data and one for the species data (ter Braak, 1995). Biplots can be produced to emphasise either samples or species or the scaling can be symmetrical.

the length of each species arrow is the multiple correlation of that species with the ordination axes; the longer arrows have the best fit to the ordination plot (ter Braak and Smilauer, 2012). For DCA, species data were log transformed using the formula y=x+1 and scaling focused on distances among response variables using biplot scaling. When interpreting biplots: species symbols in proximity to each other are those that often occur together and the distance between samples' symbols approximates the dissimilarity of their species composition (ter Braak and Smilauer, 2012).

DCA and PCA was also used to investigate the effect of environmental variables, by their inclusion as 'passive' or supplementary variables, meaning that they are plotted onto the unconstrained ordination diagram, to aid interpretation of the main axes (ter Braak and Smilauer, 2012). Centroid scores on axis 1, which represents the greatest variability, were analysed with ANOVA, to see if differences between groups were statistically significant.

#### 2.5.4.2 Classification

Classification identifies groups of quadrats (samples) with similar combinations of species and is effective at describing discontinuous variation (Hill and Smilauer, 2005). This study used WinTWINS 2.3 (the Windows version of TWINSPAN (Two-Way Indicator Species Analysis; Hill and Smilauer, 2005)[10]. Eigenvalues calculated at each division represent the size

[10] Classification results can be represented in a dendrogram, which illustrates the resulting groups and their indicator species (or 'differential' species – i.e. those species that are preferential to one

of the difference between the groups created at that division. TWINSPAN starts with a DCA analysis from which it uses the first ordination axis (i.e. that which should include most of the variability in the data) and the samples are then split into two groups at the mean of the ordination scores (Hill and Smilauer, 2005). This process is then repeated until the groups become too small to divide further.

### 2.5.5 MAVIS (Modular Analysis of Vegetation Information System)

The computer program MAVIS (Smart, 2000) was used in this study as a further method to aid comparison between the data collected from each site and the National Vegetation Classification communities (e.g. Rodwell, 1992). The program compares the collected data with the NVC constancy tables and produces a list of possible matches for the collected data. It also calculates a co-efficient of similarity as a measure of how similar the program finds the data to the chosen NVC community[11]. Its value is between 0 and 100. As previously mentioned, the NVC system used 2 m x 2 m quadrats when collecting the data, therefore, any discussion of results from MAVIS needs to take this into account.

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side of the split or the other (Hill and Smilauer, 2005)). Differential species are qualitative; therefore, TWINSPAN instead identifies a quantitative alternative, termed a pseudospecies (Hill and Smilauer, 2005). These are species found at a particular level of abundance (Hill and Smilauer, 2005), e.g. the split could be made on the basis of samples with *Centaurea nigra* at an abundance of between 26 and 50% abundance (level 3 in the default settings) and samples without *Centaurea nigra* at this particular level of abundance. These pseudospecies can be strongly associated with one side of the division or the other, in which case they are identified by the program as indicator species for these groups. In other cases, they may only be weakly associated with a group and so are identified as preferential species. These indicator and preferential species help to characterize the different groups. On dendrograms, indicator species are shown as the abbreviated species name with the level of abundance after it. Preferential species are shown in the same way but the name and level are in square brackets.

[11] Matching follows the same application of the Czekanowski coefficient as MATCH (Smart, 2000); MATCH is a program written for pre-Windows systems by Malloch (1990).

# Chapter 3

# Species-rich meadow creation using the introduction of green hay in two consecutive years

## 3.1 Introduction

Strewing species-rich green hay is an effective grassland creation technique that creates a plant community similar to that of the source site (Trueman and Millett, 2003; Donath *et al.,* 2007; Edwards *et al.,* 2007; Rydgren *et al.,* 2010; Starr-Keddle and Barrett, 2012) (Section 1.2). However, a number of characteristic grassland species fail to establish, or establish but persist poorly in re-created communities (Pywell *et al.,* 2003; Hewins *et al.,* 2012).

Pywell *et al.* (2003) analysed the results of 25 grassland creation and restoration experiments. They found that generalist species performed well, whereas other species, particularly those that are specialists of this habitat type, perform poorly. Hewins *et al.* (2012) in a review of 36 sites where species-rich grassland creation had been carried out under option HK8[12] of the Higher Level Stewardship Scheme[13] found that, although 17% of the sites could be described as good quality BAP habitat and 47% met lower definitions of BAP grassland, positive indicator species, particularly high value ones, were still lacking. It is crucial to find ways to improve the success of poorly performing species for a number of reasons, including to increase the similarity of created species-rich grasslands to 'target' semi-

[12] HK8 is the option under which species-rich grassland creation is funded.
[13] After five years of the scheme.

natural communities (Pakeman *et al.,* 2002; Smith *et al.,* 2003) and because these species may be key to food and pollinator webs in grasslands, for example, being food plants of invertebrates that are important for conservation (Pywell *et al.,* 2003). Therefore, although green hay strewing is successful in creating new species-rich grasslands, there are potential modifications to this technique.

When creating species-rich grassland on sites that are species-poor, it is essential to create opportunities for establishment in the sward, either through the creation of gaps or through the complete removal of the existing vegetation (Wells *et al.,* 1981; Bullock *et al.,* 2001; Trueman and Millett, 2003; Hofmann and Isselstein, 2004; Rayner, 2005; Edwards *et al.,* 2007). The herbicide glyphosate is often recommended as the method to achieve the removal of the existing vegetation (e.g. Wells *et al.,* 1981; Besenyei, 2000; Trueman and Millett, 2003; Flora Locale, 2005; RHS, 2017; University of Bristol, 2017; British Flora, no date; Bumblebee Conservation Trust, no date; Newcastle City Council, no date; SNH, no date), although there is also evidence that glyphosate has detrimental effects, for example, on soil micro-organisms such as arbuscular mycorrhizal fungi (AMF) (Druille *et al.,* 2015) and also on earthworms (Gaupp-Berhausen *et al.,* 2015).

This experiment investigates whether strewing species-rich hay in two consecutive years onto a species-poor grassland that has been glyphosated, introduces more species into the receiver meadow than strewing species-rich hay in just one year onto a glyphosated species-poor grassland. The hay was from the same source meadow on both occasions. Given the length of the time available for completion of this thesis and also that no published article was found with results for multiple strewing, two strews (rather than three or more strews) was chosen for this experiment. The experiment also investigates which species are difficult to transfer, in order to consider why they are more difficult and what their specific requirements might be.

Aim:

To investigate the effect on community transfer and species-richness, of strewing green hay from a species-rich source meadow, twice in consecutive years; i.e. creation of a new meadow.

Objectives:

To treat an existing grassland area of low species diversity with herbicide to remove the existing vegetation.

To create a meadow on the area by strewing green hay.

To add further hay to the created vegetation in the subsequent year from the same source site.

To compare the vegetation in the receiver meadow (before treatment, once-strewed and twice-strewed treatment areas), with the source meadow.

## 3.2 Methods

### 3.2.1 Site Descriptions

#### 3.2.1.1 Receiver meadow

Castle Vale is an area of public open space, in the north-east of Birmingham (Figures 3.1, 3.2), which consisted of unmown grassland (Figure 3.3): there are two large fields both of 1.6 ha, the easterly one of which was used for this experiment: (Castle Vale Eades; CVE). The site is owned by Birmingham City Council and is managed by their Parks Department and the Community Environment Trust (CET). The site has a varied history of use and management, although little management of the grassland has taken place recently (Table 3.1). Soil sampling was undertaken as part of the Birmingham and Black Country Nature Improvement Area Project (BBCNIA) and results were found to be in the range of fertility suitable for meadow creation (Table 3.2)[14].

In 2013, the site was dominated by grasses and had little/no diversity, with a total number of 31 species and a mean number of species per quadrat of 7.45. It had a Simpson's Index of 1.61 and, using MAVIS, most closely matched the MG1a *Arrhenatherum elatius* grassland *Festuca rubra* sub-community. The species with highest percentage frequency were *Poa pratensis*, *Plantago lanceolata* and *Festuca rubra*. *Agrostis stolonifera* and *Elytrigia repens* were each recorded in nearly a quarter of the pre-treatment quadrats and *Holcus lanatus* was recorded in more than a quarter.

[14] Soil indices in the range of +0.5 to -0.5 for total nitrogen, +2 to +3 for extractable phosphorus and values around +2 for extractable potassium are suitable for meadow creation (NIA guidelines, 2012).


Figure 3.1: Location of Castle Vale (receiver meadow) and Eades NNR (source meadow). Locations are indicated with red markers, the most northerly being Castle Vale.

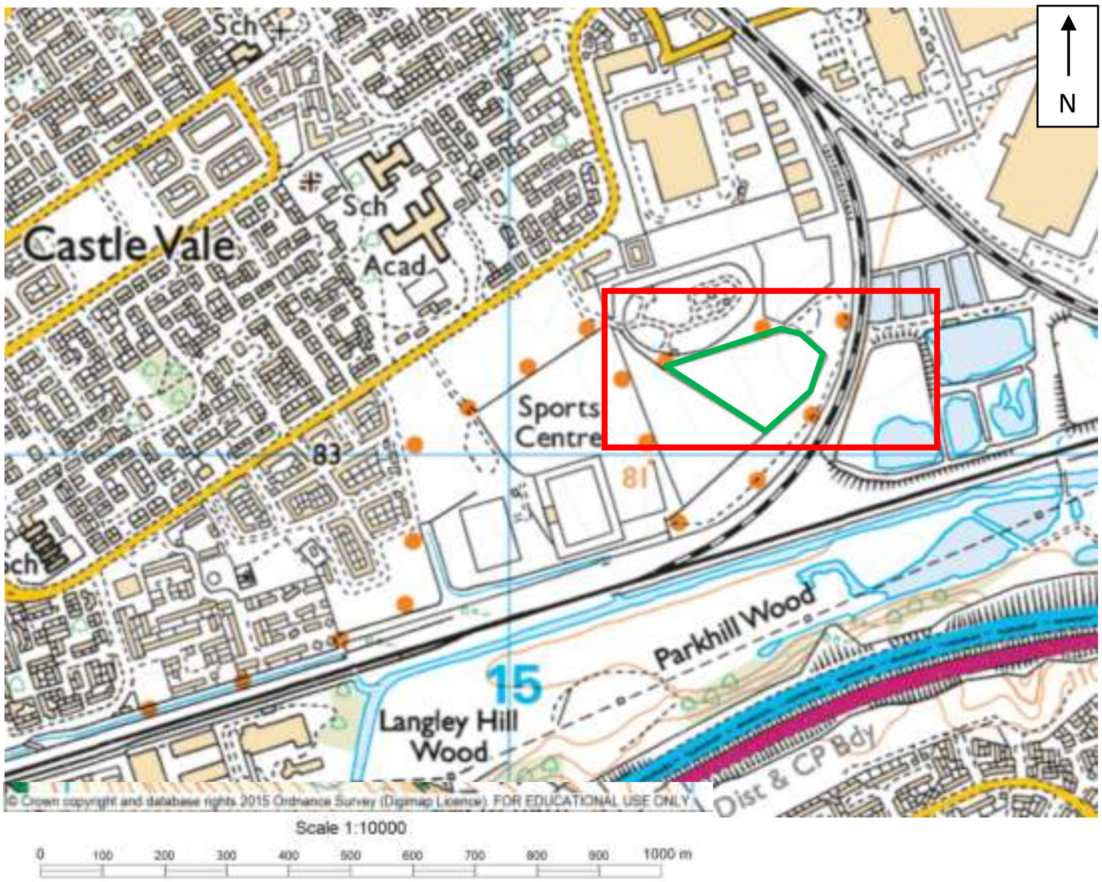



Figure 3.2: Location of Castle Vale, Birmingham, with the outline of the experimental meadow in green (Digimap, 2015).

Figure 3.3: The pre-experiment vegetation in Castle Vale grassland, facing south (2.6.2013).

Table 3.1: Site History and management (Atkins, 2007)

| Year | Site History and Management |
|---|---|
| **Pre-2013** | Used for:<br>Sewage disposal from at least 1938<br>An airfield during World War II<br>'Sewage treatment' sometime after 1963<br>A tip for incinerator residue and probably raw domestic waste in the years up to 1972 (shallow spread across the area)<br>Horse grazing pasture up to 2005<br>Soil sampling and testing undertaken by Birmingham City Council (in 2004) and Atkins (in 2006) (Atkins, 2007)<br>Atkins (2007) found that the area is constructed of made up ground (consisting of dark brown silty gravelly sand and sandy gravel) and contains a number of chemicals, mainly at safe levels, but some locations are hotspots for chemicals such as arsenic, nickel and chromium[15]<br><br>Post 2005 – there was no management regime in place |
| **2013** | June 2013: receiver meadow cut and removed and glyphosate applied. Approximately 2 weeks later the site was strewn with green hay from Eades Meadow |
| **2014** | April: thistles spot-treated with glyphosate (whole site)<br>July: Part of the field strewn with 4 more bales of green hay from Eades Meadow<br>August: some ragwort pulled and removed off site (whole site)<br>Site cut and removed (September) |
| **2015** | August and September: some ragwort pulled and removed off site (whole site)<br>Site cut and removed (September) |

[15] The potential toxicity of the soil meant that topsoil stripping was a possibility on this site. However, topsoil removal does not have a positive effect on species establishment (Kiehl *et al.,* 2006; Kardol *et al.,* 2008) is unsustainable (Hayes, 2003), damages the soil community (Kardol *et al.,* 2008) and is expensive (Klimkowska *et al.* 2010). Sub-lethal levels of toxicity can also increase diversity (Marrs, 1993; McCrea *et al.,* 2004).

Table 3.2: Results of soil sampling undertaken for the BBCNIA (Eurofins, 2013)

| **Parameter** | **Value** |
|---|---|
| Texture | Sandy, silt loam |
| pH | 6.5 |
| Total N | 0.35g/100g |
| Extractable P | 13mg/l (Index of 1) |
| Extractable K | 150mg/l (Index of 2-) |

### 3.2.1.2 Source meadow

Eades Meadow is within the nationally important group of meadows known as Foster's Green Meadows, in Worcestershire (Figures 3.1, 3.4). Eades Meadow, which is 12.5 ha, has an extremely diverse flora, including species such as *Colchicum autumnale* and *Anacamptis morio* and has developed on damp, clay soils (NE, 1981). In 2013, Eades Meadow most closely matched with the MG5b *Galium verum* sub-community and sixty-four species were recorded in the survey. Species with high percentage frequency included *Anthoxanthum odoratum, Briza media, Centaurea nigra, Lotus corniculatus, Prunella vulgaris* and *Galium verum.* Other species include *D. fuchsii* and *Neottia ovata* (Figure 3.5). The site is located approximately 16 km north-east of Worcester and 30 km south-west of the receiver meadow. Worcestershire Wildlife Trust own the site and manage it as traditional hay meadows (with a July cut and remove and aftermath grazing by cattle during the late summer and autumn; Worcestershire Wildlife Trust, 2013).


Figure 3.4: Location of Eades NNR (outlined in green), Foster's Green, Worcestershire (Digimap, 2015).

Figure 3.5: Vegetation at Eades (Fosters Green) Meadow NNR (1.7.2013).

### 3.2.1.3 Comparison of source and receiver sites

There were 47 species present at the source that were not present at the receiver, including *Rhinanthus minor, Centaurea nigra, Anthoxanthum odoratum* and four orchid species. For more details, see the results section and Table 3.5. These were the target species for this site, although the aim was also to increase the evenness of the vegetation and therefore to increase the frequency and abundance of the other appropriate meadow species present in the receiver before treatment and also present in the source meadow. Eighteen of these species are on the list of poor performing MG5 species from a review of the literature (Chapter 1, Table 1.1). Orchids are also identified in the literature as difficult species to establish in created meadows.

### 3.2.2 Experimental design

In June 2013, the receiver meadow was cut and removed and glyphosate was applied. In the fourth week of July 2013, Eades Meadow, the source, was cut and baled and the bales were transported to the receiver meadow, rolled out and spread by hand, on the same day, across the whole field. In July 2014, vegetation surveys were carried out, then, in the fourth week of July, four bales of green hay from Eades Meadow were strewn on part of the field (Figure 3.6). Unfortunately, circumstances meant that this was on top of the existing vegetation, before it had been cut. This and the timing of the green hay delivery also made it impossible to mark out plots for the second strewing and therefore there are no replications for this experiment.

Consideration of the statistical analysis of these results should bear this in mind. Transfer rates of green hay were 1:3 (source: receiver) throughout and the treatments were allocated randomly.

The experimental plot, within the meadow, was surveyed in subsequent years (Tables 3.3 and 3.4). In 2013, 20 quadrats were surveyed (before treatment) and in 2014, 40 quadrats were surveyed (all one-strew treatment, two-strews had not yet been applied). In 2015 and 2016, 60 quadrats were surveyed, 30 in the strewn once area and 30 in the strewn twice area. On every occasion there was a 5 m buffer zone from the edges of the meadow and the plots and the quadrats were located using random number co-ordinates across a grid. During these surveys any additional species, outside the quadrats, were also recorded as a species list. A walkover survey was also carried out on every survey date, walking across as much of the site as possible, in a W pattern, listing all species seen in the different treatment areas.

Table 3.3: Survey dates for Castle Vale (CVE) and its source meadow

<table>
<tr><th>Date</th><th>Meadow</th></tr>
<tr><td>1.7.13</td><td>Source meadow, Eades NNR</td></tr>
<tr><td>4.6.13</td><td>Receiver meadow (Castle Vale Eades), before treatment</td></tr>
<tr><td>20.7.14</td><td>Receiver meadow, after treatment – one-strew</td></tr>
<tr><td>30.6.15</td><td rowspan="2">Receiver meadow, after treatment – one-strew and two-strews</td></tr>
<tr><td>11.6.16</td></tr>
</table>

Table 3.4: Datasets from vegetation surveys

| Datasets from surveys | Number of quadrats |
|---|---|
| Source meadow (Eades NNR) | 50 |
| Receiver meadow (Castle Vale Eades) | |
| All quadrats 2013 – before treatment | 20 |
| All quadrats – post-treatment, 1 strew, 2014 | 40 |
| All quadrats – post-treatment, 2015 and 2016 | 60 |
| One-strew treatment | 30 |
| Two-strews treatment | 30 |

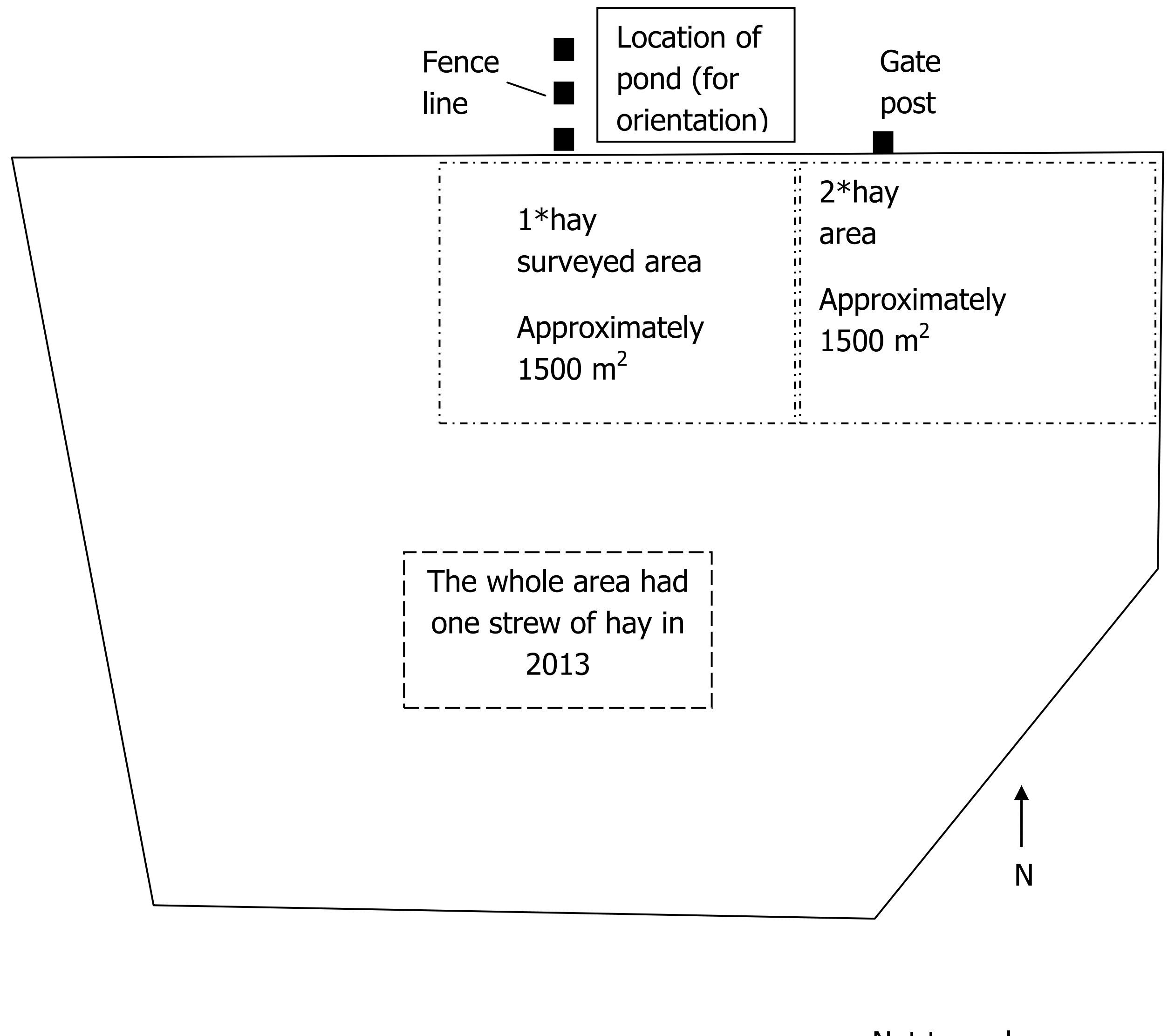



Figure 3.6: Layout of receiver meadow (Castle Vale) experimental plots.

## 3.3 Results

### 3.3.1 Comparison of species in the source and receiver meadows before and after treatment

#### 3.3.1.1 Receiver meadow baseline (CVE2013) and after treatment (CVE2014-2016)

Twenty-one species which were not found in the receiver meadow before treatment, but did occur in the source meadow, appeared in the receiver meadow post-treatment by the end of the study (i.e. were transferred; Table 3.5). Thirteen of these were recorded in the first year (2014, after one-strew, two of which were never recorded again); four more were recorded in 2015 (one in both one-and two-strew areas and three in one-strew only, one species was not recorded again); the remaining five were recorded in 2016 (three in both one- and two-strews and two in just two-strews). The 21 transferred species included 17 MG5 community species (ranging from constancy V (81-100% frequency) to I (0-20%)); 16 desirable species and five neutral species (for definitions see 2.5.1.1) and eight species from the list of poor performing species (Table 1.1, Chapter 1). The percentage frequencies of these transferred species in the source meadow, ranged from only seen on the walkover to 100%.

Twenty-six species were found in the source meadow but not in the receiver before or after treatment (i.e. were not transferred; Table 3.5). Their frequencies in the source meadow ranged from 2% to 86%. They included 14 MG5 species (constancy II to I) and four orchid species; 20 desirable species, four neutral and two undesirable species.

Table 3.5: A comparison of the species and their percentage frequencies, recorded in the source and receiver meadows in each year at Castle Vale

w/o indicates species seen on a walkover survey of the field but not recorded in a quadrat. Text in red highlights differences in presence/absence between meadows or years. D denotes desirable species, U undesirable and N species that are neither undesirable nor particularly desirable (neutral) for hay meadow communities (see 2.5.1.1). Yellow shading highlights differences between treatments. *Species on list of poor performing species, Table 1.1, Chapter 1

| | **Percentage frequencies** | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | **Source** | **Receiver** | | | | | | | | | |
| | | | | **2015** | | | **2016** | | | | |
| | **(Eades 2013)** | **CVE 2013** | **CVE 2014 (1strew)** | **CV all** | **2strews** | **1strew** | **CV all** | **2strews** | **1strew** | **MG5 Constancy** | **Desirability** |
| **3.5a Species present in source and post-treatment receiver but absent from the baseline receiver (i.e. species that were transferred by the treatment in this experiment)** | | | | | | | | | | | |
| *Alopecurus pratensis* | **2** | 0 | 0 | **2** | 0 | 3 | **0** | 0 | 0 | I | N |
| *Anthoxanthum odoratum* | **100** | 0 | **23** | 93 | 90 | 97 | 85 | 80 | 90 | IV | D |
| *Arrhenatherum elatius** | **64** | 0 | 0 | **15** | 3 | 27 | 45 | 30 | 60 | II | N |
| *Avenula pubescens** | **66** | 0 | **10** | 67 | 57 | 77 | 45 | 50 | 40 | - | N |
| *Briza media** | **90** | 0 | 0 | 0 | 0 | 0 | **12** | 17 | 7 | II | D |
| *Centaurea nigra* | **90** | 0 | 0 | 0 | 0 | 0 | **7** | 13 | 0 | IV | D |
| *Crepis biennis* | **80** | 0 | 0 | 0 | 0 | 0 | **72** | 67 | 77 | - | N |
| *Hypochaeris radicata* | **62** | 0 | **40** | 10 | 7 | 13 | 25 | 37 | 13 | III | D |
| *Lathyrus pratensis** | **14** | 0 | **5** | 0 | 0 | 0 | 0 | 0 | 0 | II | D |
| *Leontodon hispidus* | **88** | 0 | **43** | 10 | 17 | 3 | 22 | 33 | 10 | II | D |
| *Leontodon saxatile* | **14** | 0 | **10** | 0 | 0 | 0 | 0 | 0 | 0 | - | D |
| *Leucanthemum vulgare* | **86** | 0 | **43** | 30 | 43 | 17 | 47 | 60 | 33 | II | D |
| *Lotus corniculatus** | **100** | 0 | **5** | 2 | 3 | 0 | 3 | 7 | 0 | V | D |

Table 3.5: A comparison of the species and their percentage frequencies, recorded in the source and receiver meadows in each year at Castle Vale, continued

| | **Percentage frequencies** | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | **Source** | **Receiver** | | | | | | | | | |
| | | | | **2015** | | | **2016** | | | | |
| | **(Eades 2013)** | **CVE 2013** | **CVE 2014 (1strew)** | **CV all** | **2strews** | **1strew** | **CV all** | **2strews** | **1strew** | **MG5 Constancy** | **Desirability** |
| *Luzula campestris** | **16** | 0 | 0 | **2** | 0 | 3 | 7 | 0 | 13 | III | D |
| *Primula veris* | **86** | 0 | **13** | 0 | 0 | 0 | **20** | 30 | 10 | II | D |
| *Prunella vulgaris* | **94** | 0 | 0 | 0 | 0 | 0 | **10** | 20 | 0 | III | D |
| *Rhinanthus minor* | **88** | 0 | **30** | 30 | 40 | 20 | 35 | 53 | 17 | II | D |
| *Rumex acetosa** | **18** | 0 | **5** | 3 | 3 | 3 | 25 | 20 | 30 | III | D |
| *Tragopogon pratensis* | **48** | 0 | **8** | 5 | 7 | 3 | 8 | 7 | 10 | - | N |
| *Trifolium pratense** | **88** | 0 | **45** | 18 | 30 | 7 | 78 | 93 | 63 | IV | D |
| *Trisetum flavescens* | **58** | 0 | 0 | **5** | 0 | 10 | 17 | 23 | 10 | III | D |
| Mean percentage frequency | 64.38 | | | | | | | | | | |
| **3.5b Species that were never found in the receiver meadow, but were in the source** | | | | | | | | | | | |
| *Ajuga reptans* | **14** | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | - | D |
| *Allium vineale* | **18** | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | - | N |
| *Bellis perennis* | **2** | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | I | D |
| *Betonica officinalis** | **36** | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | I | D |
| *Carex flacca** | **88** | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | I | D |
| *Conopodium majus** | **12** | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | I | D |
| *Crataegus monogyna* | **2** | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | - | U |
| *Dactylorhiza fuchsii* | **46** | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | - | D |
| *Equisetum arvense* | **2** | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | - | U |
| *Filipendula ulmaria** | **34** | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | I | N |
| *Galium palustre* | **2** | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | - | D |

Table 3.5: A comparison of the species and their percentage frequencies, recorded in the source and receiver meadows in each year at Castle Vale, continued

| | **Percentage frequencies** | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | **Source** | **Receiver** | | | | | | | | | |
| | | | | **2015** | | | **2016** | | | | |
| | **(Eades 2013)** | **CVE 2013** | **CVE 2014 (1strew)** | **CV all** | **2strews** | **1strew** | **CV all** | **2strews** | **1strew** | **MG5 Constancy** | **Desirability** |
| *Galium verum** | **86** | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | II | D |
| *Hordeum secalinum* | **2** | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | - | N |
| *Linum catharticum* | **16** | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | - | D |
| *Neottia ovata* | **2** | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | - | D |
| *Medicago lupis* | **44** | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | - | D |
| *Anacamptis morio* | **24** | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | - | D |
| *Ophioglossum vulgatum** | **4** | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | I | D |
| *Ophrys apifera* | **6** | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | - | D |
| *Phleum pratense* | **6** | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | I | D |
| *Plantago media* | **2** | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | I | D |
| *Potentilla reptans** | **2** | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | I | D |
| *Schedonorus pratensis* | **52** | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | I | D |
| *Silaum silaus** | **10** | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | I | D |
| *Succisa pratensis** | **10** | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | I | D |
| *Vicia cracca** | **32** | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | I | N |
| Mean percentage frequency | 21.31 | | | | | | | | | | |
| **3.5c Species that increased substantially after treatment and were in the source** | | | | | | | | | | | |
| *Achillea millefolium* | 2 | 5 | 28 | 20 | 30 | 10 | 42 | 63 | 20 | III | D |
| *Agrostis capillaris* | 96 | 8 | 13 | 23 | 40 | 7 | 10 | 20 | 0 | IV | D |
| *Bromus hordeaceus* | 6 | 30 | 18 | 88 | 87 | 90 | 95 | 100 | 90 | I | N |
| *Cerastium fontanum* | 14 | 8 | 58 | 60 | 60 | 60 | 62 | 77 | 47 | II | D |

Table 3.5: A comparison of the species and their percentage frequencies, recorded in the source and receiver meadows in each year at Castle Vale, continued

| | **Percentage frequencies** | | | | | | | | | **MG5 Constancy** | **Desirability** |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | **Source** | **Receiver** | | | | | | | | | |
| | | | | **2015** | | | **2016** | | | | |
| | **(Eades 2013)** | **CVE 2013** | **CVE 2014 (1strew)** | **CV all** | **2strews** | **1strew** | **CV all** | **2strews** | **1strew** | | |
| *Cynosurus cristatus* | 84 | 3 | 3 | 35 | 53 | 17 | 47 | 50 | 43 | V | D |
| *Dactylis glomerata* | 44 | 10 | 8 | 3 | 0 | 7 | 28 | 27 | 30 | IV | N |
| *Festuca rubra* agg. | 84 | 58 | 25 | 72 | 63 | 80 | 95 | 97 | 93 | V | D |
| *Heracleum sphondylium* | 72 | 5 | 5 | 7 | 7 | 7 | 15 | 17 | 13 | II | D |
| *Holcus lanatus* | 92 | 35 | 78 | 92 | 87 | 97 | 97 | 97 | 97 | IV | D |
| *Lolium perenne* | 20 | 3 | 5 | 23 | 10 | 37 | 20 | 23 | 17 | III | D |
| *Plantago lanceolata* | 100 | 65 | 98 | 98 | 97 | 100 | 100 | 100 | 100 | V | D |
| *Poa trivialis* | 2 | 28 | 5 | 3 | 7 | 0 | 100 | 100 | 100 | II | D |
| *Ranunculus acris* | 38 | 5 | 40 | 20 | 33 | 7 | 58 | 73 | 43 | III | D |
| *Ranunculus bulbosus* | 18 | 3 | 10 | 2 | 0 | 3 | 18 | 30 | 7 | III | D |
| *Taraxacum* spp. | 86 | 20 | 65 | 20 | 17 | 23 | 55 | 50 | 60 | III | D |
| *Trifolium repens* | 18 | 5 | 5 | 12 | 20 | 3 | 38 | 47 | 30 | IV | D |
| Mean percentage frequency | 48.50 | | | | | | | | | | |
| **3.5d Species that decreased substantially after treatment and were in the source** | | | | | | | | | | | |
| *Scorzoneroides autumnalis* | 24 | 3 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | III | D |
| **3.5e Species present in the receiver before treatment that were not present in the source** | | | | | | | | | | | |
| *Agrostis stolonifera* | 0 | 23 | **0** | 0 | 0 | 0 | 0 | 0 | 0 | I | U |
| *Cardamine hirsuta* | 0 | 18 | 3 | **0** | 0 | 0 | 0 | 0 | 0 | - | U |
| *Cirsium arvense* | 0 | 28 | 10 | 2 | 0 | 3 | 10 | 17 | 3 | II | U |
| *Cirsium vulgare* | 0 | 18 | 70 | 47 | 60 | 33 | 18 | 27 | 10 | - | U |
| *Elytrigia repens* | 0 | 23 | 8 | **0** | 0 | 0 | 0 | 0 | 0 | - | U |

Table 3.5: A comparison of the species and their percentage frequencies, recorded in the source and receiver meadows in each year at Castle Vale, continued

| | **Percentage frequencies** | | | | | | | | | **MG5 Constancy** | **Desirability** |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | **Source** | **Receiver** | | | | | | | | | |
| | | | | **2015** | | | **2016** | | | | |
| | **(Eades 2013)** | **CVE 2013** | **CVE 2014 (1strew)** | **CV all** | **2strews** | **1strew** | **CV all** | **2strews** | **1strew** | | |
| *Epilobium ciliatum* | 0 | 15 | 88 | 2 | 3 | 0 | 2 | 0 | 3 | - | U |
| *Galium aparine* | 0 | 3 | **0** | **2** | 3 | 0 | **0** | 0 | 0 | - | U |
| *Geranium dissectum* | 0 | 13 | 10 | 37 | 37 | 37 | 60 | 70 | 50 | - | N |
| *Poa pratensis* | 0 | 65 | **0** | 88 | 83 | 93 | **0** | 0 | 0 | II | D |
| *Quercus* sp. (seedling) | 0 | 3 | **0** | 0 | 0 | 0 | 0 | 0 | 0 | - | U |
| *Ranunculus repens* | 0 | 3 | **0** | 0 | 0 | 0 | 0 | 0 | 0 | I | U |
| *Rumex crispus* | 0 | 3 | **0** | **2** | 3 | 0 | **0** | 0 | 0 | - | U |
| *Rumex* sp. | 0 | 3 | **0** | 0 | 0 | 0 | 0 | 0 | 0 | - | U |
| *Senecio jacobaea* | 0 | 13 | 60 | 30 | 33 | 27 | 55 | 80 | 30 | I | U |
| *Veronica* sp. | 0 | 5 | 23 | **0** | 0 | 0 | 0 | 0 | 0 | - | N |
| *Vicia sativa* | 0 | 43 | 10 | 20 | 20 | 20 | 63 | 70 | 57 | - | N |
| **3.5f Species present in post-treatment receiver that were not recorded before treatment nor in the source** | | | | | | | | | | | |
| *Aphanes arvensis* | 0 | 0 | **13** | 2 | 3 | 0 | **0** | 0 | 0 | - | N |
| *Anisantha sterilis* | 0 | 0 | 0 | 0 | 0 | 0 | **3** | 3 | 3 | - | U |
| *Chamerion angustifolium* | 0 | 0 | **3** | 0 | 0 | 0 | 0 | 0 | 0 | - | U |
| *Conyza* sp. | 0 | 0 | **13** | 0 | 0 | 0 | 0 | 0 | 0 | - | U |
| *Crepis capillaris* | 0 | 0 | **73** | 0 | 0 | 0 | 53 | 73 | 33 | I | D |
| *Crepis* sp. | 0 | 0 | **50** | 72 | 80 | 63 | **0** | 0 | 0 | - | N |
| *Crepis vesicara* | 0 | 0 | 0 | **68** | 77 | 60 | 50 | 63 | 37 | - | N |
| Brassicaceae sp. | 0 | 0 | 0 | **2** | 3 | 0 | 0 | 0 | 0 | - | U |
| *Epilobium hirsutum* | 0 | 0 | **35** | 2 | 3 | 0 | **0** | 0 | 0 | - | U |

Table 3.5: A comparison of the species and their percentage frequencies, recorded in the source and receiver meadows in each year at Castle Vale, continued

| | Percentage frequencies | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Source | Receiver | | | | | | | | | | |
| | | | | | 2015 | | | | 2016 | | | |
| | (Eades 2013) | CVE 2013 | CVE 2014 (1strew) | | CV all | 2strews | 1strew | | CV all | 2strews | 1strew | MG5 Constancy | Desirability |
| *Filago germanica* | 0 | 0 | 0 | | **2** | 3 | 0 | | **0** | 0 | 0 | - | U |
| *Fraxinus excelsior* (seedling) | 0 | 0 | **3** | | **0** | 0 | 0 | | 0 | 0 | 0 | - | U |
| *Geum urbanum* | 0 | 0 | **8** | | **0** | 0 | 0 | | 0 | 0 | 0 | - | U |
| *Lactuca serriola* | 0 | 0 | **3** | | 2 | 0 | 3 | | **0** | 0 | 0 | - | U |
| *Rubus fruticosus* agg. | 0 | 0 | 0 | | **2** | 3 | 0 | | 2 | 3 | 0 | - | U |
| *Rumex sanguineus* | 0 | 0 | 0 | | 0 | 0 | 0 | | **5** | 10 | 0 | - | U |
| *Senecio vulgaris* | 0 | 0 | **5** | | **0** | 0 | 0 | | 0 | 0 | 0 | - | U |
| *Sisymbrium officinale* | 0 | 0 | **18** | | **0** | 0 | 0 | | 0 | 0 | 0 | - | U |
| *Sonchus asper* | 0 | 0 | **53** | | 5 | 10 | 0 | | **0** | 0 | 0 | - | U |
| *Trifolium dubium* | 0 | 0 | **35** | | 22 | 27 | 17 | | 93 | 93 | 93 | II | D |
| *Urtica dioica* | 0 | 0 | **3** | | **0** | 0 | 0 | | 0 | 0 | 0 | - | U |
| *Veronica chamaedrys* | 0 | 0 | 0 | | **5** | 0 | 10 | | 5 | 10 | 0 | II | D |
| *Vicia hirsuta* | 0 | 0 | **15** | | 42 | 40 | 43 | | 73 | 67 | 80 | - | N |
| Bare ground | 0 | 63 | 100 | | 20 | 17 | 23 | | 2 | 0 | 3 | - | - |

Twenty-two other species that had not been recorded before were found in the receiver meadow after treatment, but these were also not in the source meadow (Table 3.5). Fifteen of these were recorded in 2014 (after one-strew, nine of which were not recorded again and five more of which were not recorded after 2015); five more were recorded in 2015 (one in both one-strew and two-strew, three in two-strews only and one in one-strew only, two of which were not recorded again) and the remaining two were first recorded in 2016 (one in both one-strew and two-strews and one in two-strews only). Of these 22 species, 15 were undesirable, three were desirable species and three were neutral.

All of the species that were present in the source but were already in the receiver before treatment (17 species), except *Scorzoneroides autumnalis* (which was recorded as 3% frequency before treatment and disappeared post-treatment), subsequently increased substantially post-treatment in both the one-strew and two-strew areas.  Their percentage frequency in the source ranged from 2% to 96%. It should be noted that the meadow had been treated with herbicide to kill off the existing vegetation, so all the post-treatment vegetation should have been new, although it could also include species from the seedbank.

### 3.3.1.2 One-strew and two-strew treatment areas

Nine species were only recorded in the one-strew area in either 2015 or 2016, three of which (one undesirable, one neutral and one desirable) were never recorded in the two-strews area. Four of the nine were desirable species (plus one neutral, MG5 species; Table 3.6). Nine species were only recorded in the two-strews area and were never recorded in the one-strew area, two of which were desirable (*C. nigra* and *P. vulgaris*) and neither of which were recorded before treatment, making them of particular interest (Table 3.5a; Table 3.7). *Lathyrus pratensis, Luzula campestris* and *Dactylis glomerata* (present in the baseline receiver, but increased after treatment) are all mentioned the list of poor performing species in Chapter 1 (Table 1.1).

In 2015, 24 species were recorded at a substantially higher frequency in the two-strews area compared to the one-strew area, 11 of which were desirable. These included: *L. hispidus, L. vulgare, L. corniculatus, R. minor T. pratensis* and *T. pratense,* none of which were present before treatment, and were therefore particular target species (Table 3.5a). In 2016, 29 species were recorded at a substantially higher frequency in the two-strew area compared to the one-strew area, 20 of which were desirable. *B. media, C. nigra, L. hispidus, L. vulgare, L. corniculatus, P. veris, P. vulgaris, R. minor, T. pratense* and *T. flavescens* were not present before treatment (Table 3.5a). Twelve species were recorded at a higher frequency in the two-strew area in both of these years.

In 2015, 10 species were recorded at a substantially higher frequency in the one-strew area compared to the two-strews area, seven of which were desirable species. These included: *A. pratensis, L. campestris* and *T. flavescens,* none of which were present before treatment, and were therefore particular target species (Table 3.5a). In 2016, seven species were recorded at a considerably higher frequency in the one-strew area compared to the two-strews area, three of which were desirable species. *L. campestris* was not present before treatment (Table 3.5a). *A. elatius* and *L. campestris* were recorded at a higher frequency in the one-strew area in both years. Bare ground was also recorded at a higher frequency in the one-strew area in both years.

Table 3.6 Species that were recorded only in the 1-strew treatment area in 2014, 2015 or 2016

| | **Percentage frequencies** | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | | **2015** | | | | **2016** | | |
| | **CVE2014** | | **CVall** | **2strews** | **1strew** | | **CV all** | **2strews** | **1strew** |
| *Alopecurus pratensis* | 0 | | **2** | 0 | 3 | | **0** | 0 | 0 |
| *Lathyrus pratensis** † | **5** | | 0 | 0 | 0 | | 0 | 0 | 0 |
| *Leontodon saxatile** | **10** | | 0 | 0 | 0 | | 0 | 0 | 0 |
| *Luzula campestris* † | 0 | | **2** | 0 | 3 | | 7 | 0 | 13 |
| *Trisetum flavescens* | 0 | | **5** | 0 | 10 | | 17 | 23 | 10 |
| *Dactylis glomerata* † | 8 | | 3 | 0 | 7 | | 28 | 27 | 30 |
| *Ranunculus bulbosus* | 10 | | 2 | 0 | 3 | | 18 | 30 | 7 |
| *Cardamine hirsuta** | 3 | | **0** | 0 | 0 | | 0 | 0 | 0 |
| *Elytrigia repens* | 8 | | **0** | 0 | 0 | | 0 | 0 | 0 |
| *Cirsium arvense* | 10 | | 2 | 0 | 3 | | 10 | 17 | 3 |
| *Epilobium ciliatum* | 88 | | 2 | 3 | 0 | | 2 | 0 | 3 |
| *Veronica* sp.* | 23 | | **0** | 0 | 0 | | 0 | 0 | 0 |
| *Lactuca serriola* | **3** | | 2 | 0 | 3 | | **0** | 0 | 0 |

* Species that were recorded only in 2014 (when there was only the 1-strew treatment)
† MG5 species identified as performing poorly from a review of the literature, (Chapter 1, Table 1.1).

Table 3.7 Species that were only ever recorded in the 2-strews treatment area*

| | Percentage frequencies | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | | 2015 | | | | 2016 | | |
| | CVE2014 | | CVall | 2strews | 1strew | | CVall | 2strews | 1strew |
| *Centaurea nigra* | 0 | | 0 | 0 | 0 | | **7** | 13 | 0 |
| *Lotus corniculatus*[†] | **5** | | 2 | 3 | 0 | | 3 | 7 | 0 |
| *Prunella vulgaris* | 0 | | 0 | 0 | 0 | | **10** | 20 | 0 |
| *Rumex crispus* | **0** | | **2** | 3 | 0 | | **0** | 0 | 0 |
| *Aphanes arvensis* | **13** | | 2 | 3 | 0 | | **0** | 0 | 0 |
| *Filago germanica* | 0 | | **2** | 3 | 0 | | **0** | 0 | 0 |
| *Veronica chamaedrys* | 0 | | **5** | 0 | 10 | | 5 | 10 | 0 |

Note: Additional to these species, *Agrostis capillaris* was recorded at much higher frequency in 2-strews in 2015 and was only recorded in 2-strews in 2016

*In 2015 or 2016

[†]MG5 species identified as performing poorly from a review of the literature, (Chapter 1, Table 1.1).

### 3.3.1.3 Significance testing of treatment effects

A factorial repeated measures ANOVA was conducted to determine the effects of year (2) and haying (2) levels on the mean percentage cover of the desirable species (following Laerd Statistics (2013)). The interaction between haying and year was not significant [$F(1,31) = 3.719$ , $p = 0.063$]. There was a significant effect of year [$F(1,31) = 4.595$, $p = 0.040$], the second year having a higher mean percentage cover of desirable species (mean: 0.47, (95% CI, 0.19 to 1.12)) than the first year (mean: 0.16, (95% CI, 0.06 to 0.46)) . The effect of haying frequency was not significant, $F(1, 31) = 3.779$, $p = 0.061$, although there was a marked difference between the two levels of the treatment, with two years of haying having a higher mean percentage cover of the desirable species (0.37 (95% CI, 0.16 to 0.87)) than a single year

(0.20 (95% CI, 0.08 to 0.51)). The mean values are low due to a high number of zeros in the data.

### 3.3.2 Comparisons of total number of species per site and species-richness for each quadrat

Of all the datasets, the source meadow (Eades) had the highest total number and mean number of species per quadrat (Tables 3.8 and 3.9). Of the receiver meadow (CVE) groups, CVE2016 two-strews had the highest mean number of species per quadrat and CVE2014 had the highest total number of species, although this included a high number of undesirable/non-meadow species. CVE2016all had the highest total number of desirable species. CVE2013 had the lowest total number and mean number of species per quadrat. CVE two-strews had a higher total number and mean number of species per quadrat than CVE one-strew in both years, when considering all species. However, when considering only desirable species, for total number of species, CVE one-strew was higher in 2015, although when considering desirable plus neutral species, CVE two-strews was higher in both years. CVE two-strews had a higher number of undesirable species in both years. CVE2014 had the highest total number of undesirable species and these generally decreased over time. CVE2013 had the highest mean number of undesirable species.

Table 3.8: Total number of species per quadrat for CVE and its source

| **Meadow/treatment** | **Year of survey** | | | |
|---|---|---|---|---|
| | **2013** | **2014** | **2015** | **2016** |
| Source meadow (Eades) | 64 | N/A | N/A | N/A |
| Receiver (CVE): | | | | |
| All quadrats | 31 | As 1strew | 51 | 48 |
| CVE 2strews | N/A | N/A | 43 | 46 |
| CVE 1strew | N/A | 53 | 40 | 34 |
| **Desirable species only** | | | | |
| Source meadow (Eades) | 52 | N/A | N/A | N/A |
| Receiver (CVE) | | | | |
| All quadrats | 15 | As 1strew | 28 | 31 |
| CVE 2strews | N/A | N/A | 24 | 30 |
| CVE 1strew | N/A | 27 | 25 | 26 |
| **Neutral species only** | | | | |
| Source meadow (Eades) | 11 | N/A | N/A | N/A |
| Receiver (CVE): | | | | |
| All quadrats | 6 | As 1strew | 11 | 10 |
| CVE 2strews | N/A | N/A | 9 | 10 |
| CVE 1strew | N/A | 9 | 11 | 10 |
| **Undesirable species only** | | | | |
| Source meadow (Eades) | 3 | N/A | N/A | N/A |
| Receiver (CVE): | | | | |
| All quadrats | 9 | As 1strew | 12 | 7 |
| CVE 2strews | N/A | N/A | 10 | 6 |
| CVE 1strew | N/A | 14 | 4 | 5 |

Table 3.9: Mean number of species per quadrat for CVE and its source

| | **Year** | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| **Meadow/treatment** | **2013** | | **2014** | | **2015** | | **2016** | |
| | Mean | s.e. | Mean | s.e. | Mean | s.e. | Mean | s.e. |
| Source meadow (Eades) | 27.06 | 0.37 | N/A | N/A | N/A | N/A | N/A | N/A |
| Receiver (CVE): | | | | | | | | |
| All quadrats | 7.45 | 0.44 | As 1strew | | 13.22 | 0.37 | 19.35 | 0.50 |
| CVE 2strews | N/A | N/A | N/A | N/A | 14.03 | 0.49 | 21.97 | 0.54 |
| CVE 1strew | N/A | N/A | 13.45 | 0.69 | 12.40 | 0.51 | 16.73 | 0.53 |
| **Desirable species only** | | | | | | | | |
| Source meadow (Eades) | 23.06 | 0.31 | N/A | N/A | N/A | N/A | N/A | N/A |
| Receiver (CVE): | | | | | | | | |
| All quadrats | 3.50 | 0.23 | As 1strew | | 8.07 | 0.30 | 13.00 | 0.44 |
| CVE 2strews | N/A | N/A | N/A | N/A | 8.70 | 0.47 | 15.07 | 0.56 |
| CVE 1strew | N/A | N/A | 8.03 | 0.62 | 7.43 | 0.35 | 10.93 | 0.44 |
| **Neutral species only** | | | | | | | | |
| Source meadow (Eades) | 3.96 | 0.16 | N/A | N/A | N/A | N/A | N/A | N/A |
| Receiver (CVE): | | | | | | | | |
| All quadrats | 1.55 | 0.19 | As 1strew | | 4.18 | 0.18 | 6.67 | 0.18 |
| CVE 2strews | N/A | N/A | N/A | N/A | 4.07 | 0.23 | 6.67 | 0.28 |
| CVE 1strew | N/A | N/A | 1.04 | 0.19 | 4.30 | 0.27 | 6.67 | 0.23 |
| **Undesirable species only** | | | | | | | | |
| Source meadow (Eades) | 0.04 | 0.03 | N/A | N/A | N/A | N/A | N/A | N/A |
| Receiver (CVE): | | | | | | | | |
| All quadrats | 1.80 | 0.16 | As 1strew | | 0.97 | 0.11 | 0.95 | 0.12 |
| CVE 2strews | N/A | N/A | N/A | N/A | 1.27 | 0.17 | 0.82 | 0.15 |
| CVE 1strew | N/A | N/A | 1.18 | 0.21 | 0.67 | 0.11 | 0.50 | 0.13 |

### 3.3.3 Species diversity and similarity measures

CVE2013 had the lowest diversity and evenness and the highest dominance of all the datasets (Table 3.10). CVE2015 two-strews had the highest diversity and evenness and the lowest dominance. By 2016, two-strews was marginally more similar to the source meadow than was the one-strew treatment (Figure 3.7).

Table 3.10: Species diversity measures for CVE and Eades

| | **Simpson's Index** | **Simpson's Measure of Evenness** | **Berger-Parker** | **Species with the highest total percentage cover** |
|---|---|---|---|---|
| Ea2013 (source) | 2.75 | 0.24 | 0.18 | *Lot cor* |
| CVE2013 (baseline receiver) | 1.61 | 0.16 | 0.41 | *Pla lan* |
| **Receiver after treatment:** | | | | |
| CVE2014 | 2.38 | 0.20 | 0.18 | *Epi cil* |
| CVE2015 all | 2.42 | 0.22 | 0.14 | *Hol lan* |
| CVE2015 2strews | 2.66 | 0.33 | 0.11 | *Bro hor* |
| CVE2015 1strew | 2.16 | 0.22 | 0.17 | *Hol lan* |
| CVE2016 all | 2.54 | 0.25 | 0.16 | *Hol lan* |
| CVE2016 2strews | 2.62 | 0.29 | 0.15 | *Hol lan* |
| CVE2016 1strew | 2.18 | 0.25 | 0.19 | *Hol lan* |

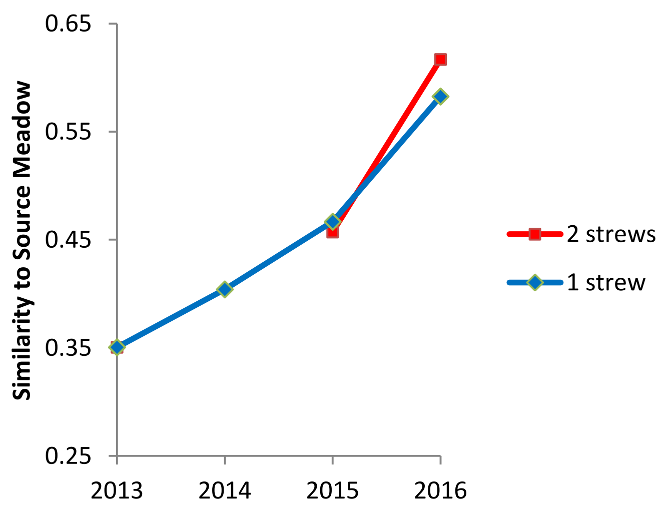



Figure 3.7: Comparison of source and receiver meadow treatments using the Czekanowski coefficient.

### 3.3.4 Comparison with NVC communities

In 2013, the source meadow (Ea2013) most closely matched with the MG5b *Galium verum* sub-community (Table 3.10). The baseline receiver (CVE2013) matched the MG1a *Arrhenatherum elatius* grassland *Festuca rubra* sub-community most closely, but by 2016, all groups most closely matched with an MG5 community type (either MG5a *Lathyrus pratensis* sub-community or MG5). The overall trend was for a closer match to MG5 over time and for two-strews to have a closer match (to MG5) than one-strew (Tables 3.11 and 3.12).

Table 3.11: Similarity to NVC communities and sub-communities, expressed as highest three coefficients from MAVIS analysis

| **Receiver (CVE2013)** | **Source (Ea2013)** | |
|---|---|---|
| MG1a 47.02<br>SD8a 42.94<br>MG11 42.00 | MG5b 65.41<br>MG5 65.00<br>MG5a 64.20 | |
| **CVE2014 all** | **CVE2015 all** | **CVE2016 all** |
| OV23 47.62<br>MG5a 47.47<br>MG5 46.49 | MG6b 51.65<br>MG6 50.20<br>MG1a 49.35 | MG5a 58.85<br>MG5 58.13<br>MG5b 54.71 |
| **CVE2015 one-strew** | **CVE2015 two-strews** | |
| MG6b 53.89<br>MG6 52.46<br>MG1a 51.45 | MG6b 50.78<br>MG6 49.20<br>MG5a 47.36 | |
| **CVE2016 one-strew** | **CVE2016 two-strews** | |
| MG5 55.39<br>MG5a 55.35<br>MG1e 52.06 | MG5a 60.12<br>MG5 58.66<br>MG5b 56.08 | |

Table 3.12: Closest matches to an MG5 community type from MAVIS for the receiver meadow

| | 2013 | 2014 | | 2015 | | 2016 | |
|---|---|---|---|---|---|---|---|
| CVE all | No match | 47.47 | MG5a | 48.57 | MG5a | 58.85 | MG5a |
| CVE two-strews | N/A | N/A | | 47.36 | MG5a | 60.12 | MG5a |
| CVE one-strew | N/A | N/A | | 47.28 | MG5a | 55.39 | MG5 |

Figures in red are not the closest match to a NVC type, for closest matches see Table 3.11.

### 3.3.5 TWINSPAN

The results of a TWINSPAN analysis of the combined data from the receiver meadow and its source are presented as a dendrogram (Figure 3.8). The source meadow (Eades) quadrats were initially included in the analysis, but these were all separated off from the receiver meadow (CVE) quadrats at the first division and so are excluded from the dendrogram.

At the next division, CVE2015 (except for 3 quadrats), CVE2016 and three of the CVE2013 quadrats (group 010) are divided from CVE2014, the rest of CVE2013 (baseline receiver) and three CVE2015 quadrats. Group 010 is on the Eades side of the diagram and so is most similar to the source meadow. The indicator species of group 010 are *Bromus hordeaceus* and *Anthoxanthum odoratum* and that of group 011 is *Epilobium ciliatum.*

The last division results in group 01000, containing quadrats from 2016, from both one-strew and two-strews treatments and one from 2013; group 01001, which contains the remaining quadrats from 2016; group 01010, which contains all except three quadrats from 2015; group 01001, which contains just two quadrats, from 2013; groups 01100 and 01101, which contain a mix of 2013, 2015 and some 2014 quadrats and groups 01110 and 01111, which both contain a small number of 2014 quadrats. The eigenvalues are very low throughout the analysis, indicating that there is little difference between the groups. Group 01000, which contains the majority of the 2016 quadrats and the majority of the 2016 two-strews quadrats, is the group most similar to the source meadow (Eades) quadrats. Group 01111, which contains the majority of the baseline receiver quadrats, is the least similar. The 2013 quadrats are generally found on the right of the diagram (least similar to the source, the majority being in groups 01111 and 01110) and when moving from right to left, the 2014 quadrats are generally found next (groups  01101 and 01100), then the 2015 quadrats (group 01010), then the 2016 quadrats (groups 0100 and 01000). The indicator species for group 01000, i.e. that most similar to the source, are *Holcus lanatus* and *Arrhenatherum elatius*.

3. One-strew and two-strews (Castle Vale and Eades)

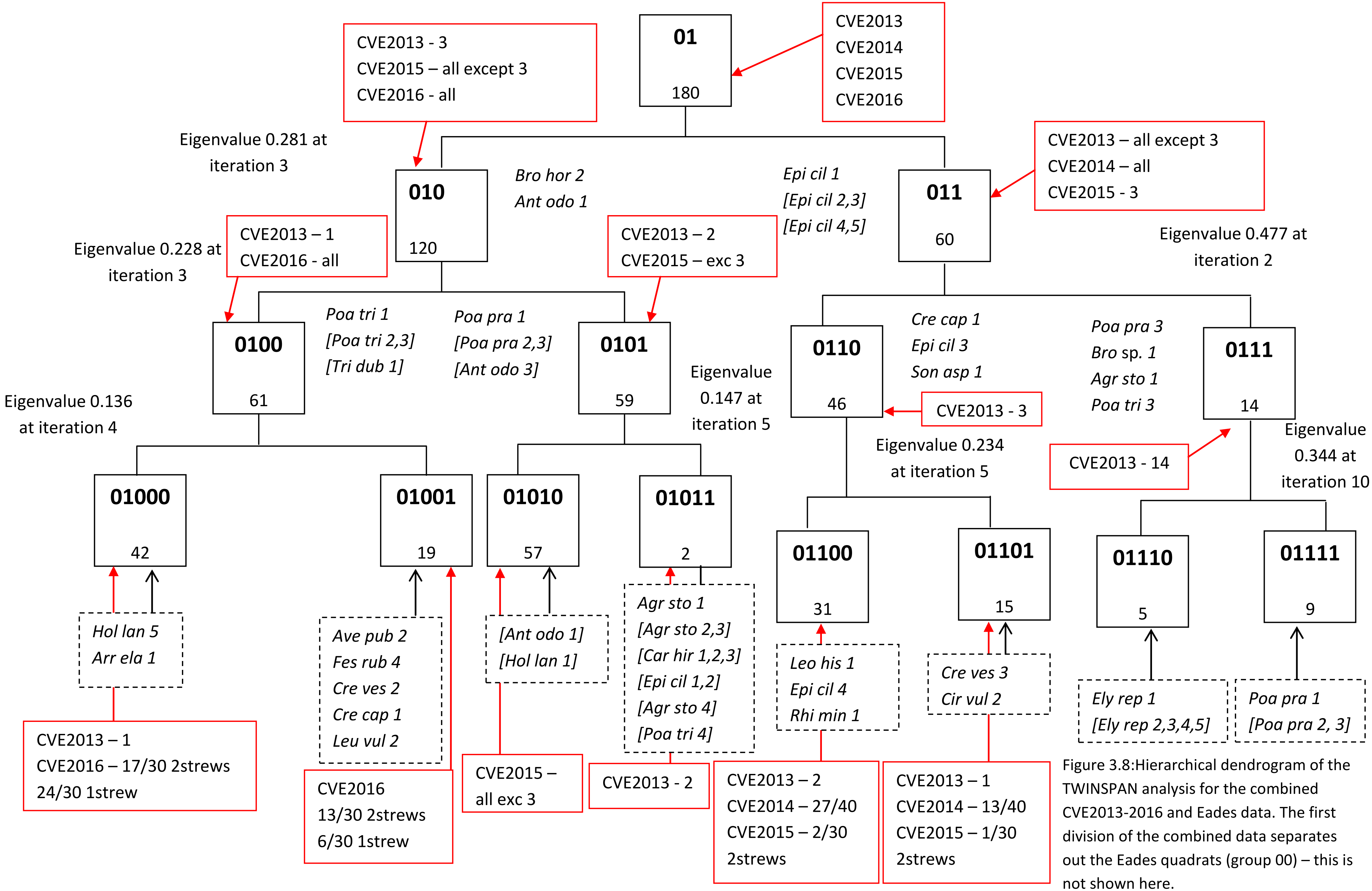



Figure 3.8:Hierarchical dendrogram of the TWINSPAN analysis for the combined CVE2013-2016 and Eades data. The first division of the combined data separates out the Eades quadrats (group 00) – this is not shown here.

### 3.3.6 Detrended Correspondence Analysis (DCA)

A Detrended Correspondence Analysis (DCA) was performed on the combined CVE and Eades data, as the gradient length was 4.1SD. A DCA ordination samples plot of the data shows clear separation of the source quadrats from all years of the receiver meadow quadrats (Figure 3.9). The baseline receiver quadrats are the most widely spread, on both axes, and the source meadow quadrats are the most tightly clustered (on both axes) and are associated with the highest diversity of species (and also the desirable hay meadow species; Figure 3.10). The quadrats in each of the years 2014-16 are more tightly clustered than 2013 (Figure 3.9). There is little overlap of the 2014 quadrats with any other groups. The 2015 and 2016 quadrats are possibly slightly closer to the source meadow quadrats than the previous years' on axis 1; the two-strews quadrats being slightly closer than the one-strew quadrats, on axis 1. This is also illustrated by the DCA samples plot with the quadrats coded as environmental variables and shown as centroids (mean of the quadrats ordination scores on each axis) (Figure 3.11).

In addition, the centroids plot (Figure 3.11) shows more clearly the separation between the source and the baseline receiver (CVE2013) on axis 1, with some separation on axis 2. The 2014 quadrats are substantially distant from the baseline receiver (CVE2013) on axis 2 and are below the point at which the source centroid is located on axis 2. However, the samples from 2015 and 2016 are on a direct line between the source meadow centroid and the baseline. The two-strews centroid is closer to the source than the one-strew centroid in both years and the 2016 centroids are closer to the source than their equivalent 2015 centroid.

A one-way ANOVA on the axis 1 ordination scores of the centroids showed that these differences were statistically significant [$F(6, 223) = 28.314$, $p < 0.001$]. *Post hoc* Tamhane tests showed that the centroid scores for CVE2016 two-strews and CVE2016 one-strew were not significantly different to each other or to the source meadow (Ea2013) on axis 1 (homogenous subset 'b'), but were significantly different to all other groups (Table 3.13). CVE2014, CVE2015 one-strew and CVE2015 two-strews were also not significantly different to each other, but were to all other groups (homogenous subset 'c').

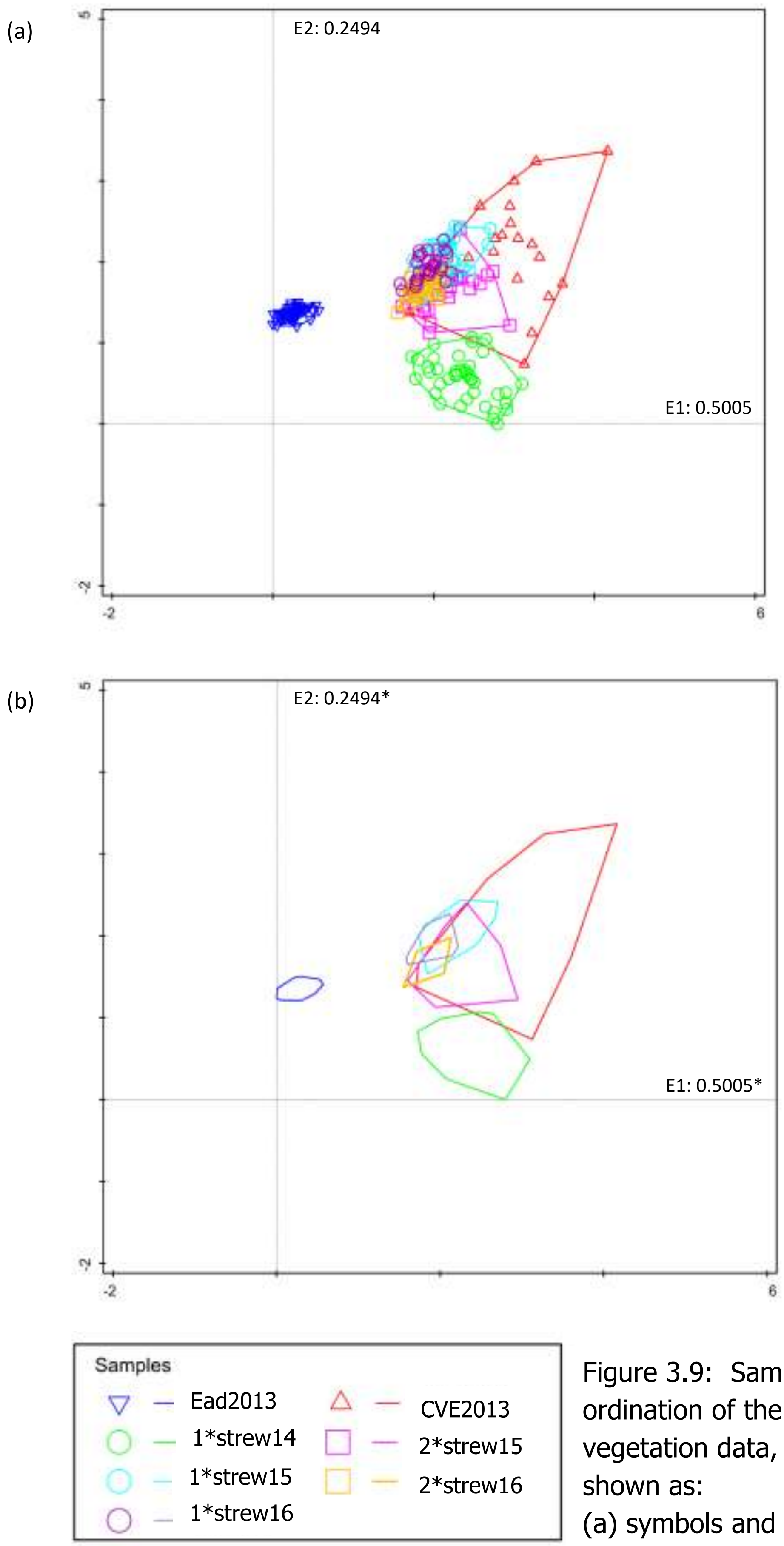
Table 3.13: Results of comparisons of Canoco DCA axis 1 centroid ordination scores for CVE and Eades

| **Dataset** | **Mean** | **S.E.** | **df** | **MS** | ***F*** | **p** |
|---|---|---|---|---|---|---|
| CVE2013 (baseline receiver) | 0.2718[a] | 0.031 | 6, 223 | 13.384 | 28.314 | <0.001*** |
| Ea2013 (source) | 1.3293[b] | 0.197 | | | | |
| CVE2016 2strews | 1.8701[b] | 0.028 | | | | |
| CVE2016 1strew | 1.9337[b] | 0.029 | | | | |
| CVE2015 2strews | 2.1726[c] | 0.055 | | | | |
| CVE20151strew | 2.1743[c] | 0.040 | | | | |
| CVE2014 | 2.3807[c] | 0.053 | | | | |

*** indicates the significance levels when p values are p <0.001. Treatment means with the same label (a-c) are not significantly different from one another (Tamhane; p <0.05).

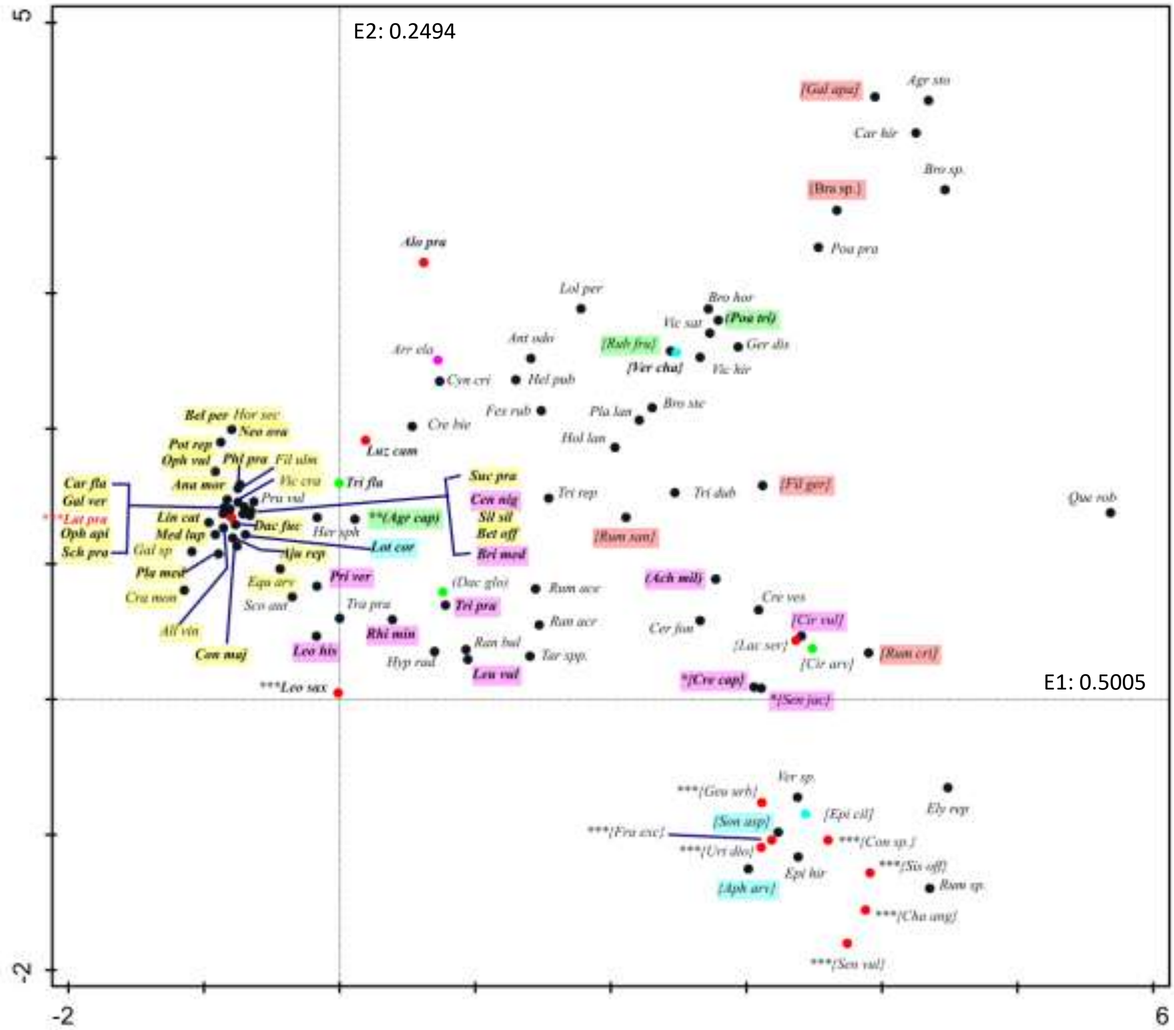
The DCA species ordination plot (Figure 3.10) shows that there are some undesirable weeds with high and low ordination scores on axis 2, which probably accounts for the separation of the quadrats on this axis. Species with high scores on both axes are associated with the before treatment quadrats. Low axis 1 scoring species are associated with the source meadow (Eades) quadrats and are mostly species that have not transferred. Species that were only ever found in two-strews or that were recorded at a higher percentage frequency in two-strews have positive axis 2 scores. Species with high axis 1 scores are associated with the quadrats from 2014 and seem to be common agricultural weeds which came in at creation, but were subsequently lost.

Figure 3.12 shows the DCA samples plot with the quadrats coded as their TWINSPAN group. The TWINSPAN division first splits of the source meadow quadrats from the rest, with an eigenvalue of 0.452 – suggesting that the receiver meadow quadrats are not yet similar to the source, as can be seen in the DCA plot (Figure 3.12). The least similar TWINSPAN groups to the source meadow, on both axes, are group 01111, which contains nine CVE2013 quadrats; then groups 01011 (two more CVE2013 quadrats) and 01110 (five more CVE2013 quadrats). The most similar group, on both axes, is probably group 01001, which comprises 13 two-strews quadrats and six one-strew quadrats, all from CVE2016.




*E1: Eigenvlaue for axis 1;
E2: Eigenvalue for axis 2

Figure 3.9: Samples plot of the DCA ordination of the Castle Vale and Eades vegetation data, quadrats (samples) shown as:
(a) symbols and envelopes and
(b) envelopes only.




Key

- Species only ever found in 1strew
- Species only ever found in 2strews
- Species found at higher percentage frequency in 1strew
- Species found at higher percentage frequency in 2strews
- Species found in 1strew only in either 2015 or 2016 and both treatments in the other year
- Species found in 2strews only in either 2015 or 2016 and both treatments in the other year
- Species found only in 1strew in either 2015 or 2016 and only in 2strews in the other year
- Species found in 1strew in 2014 and in 2 strews only in following year(s)
- Species that did not transfer

*Species only present in 2016; **Species also found more of in 2 strews than 1 strew; ***Species only found in 2014 1 strew; Species in **bold** are desirable species; Species colour coded but no brackets – species not in CVE before treatment; ( ) – species in source and CVE before treatment; [ ] Species not in source, but in receiver pre- and post-treatment;

Figure 3.10: Species graph of the DCA ordination of the Castle Vale and Eades vegetation data.

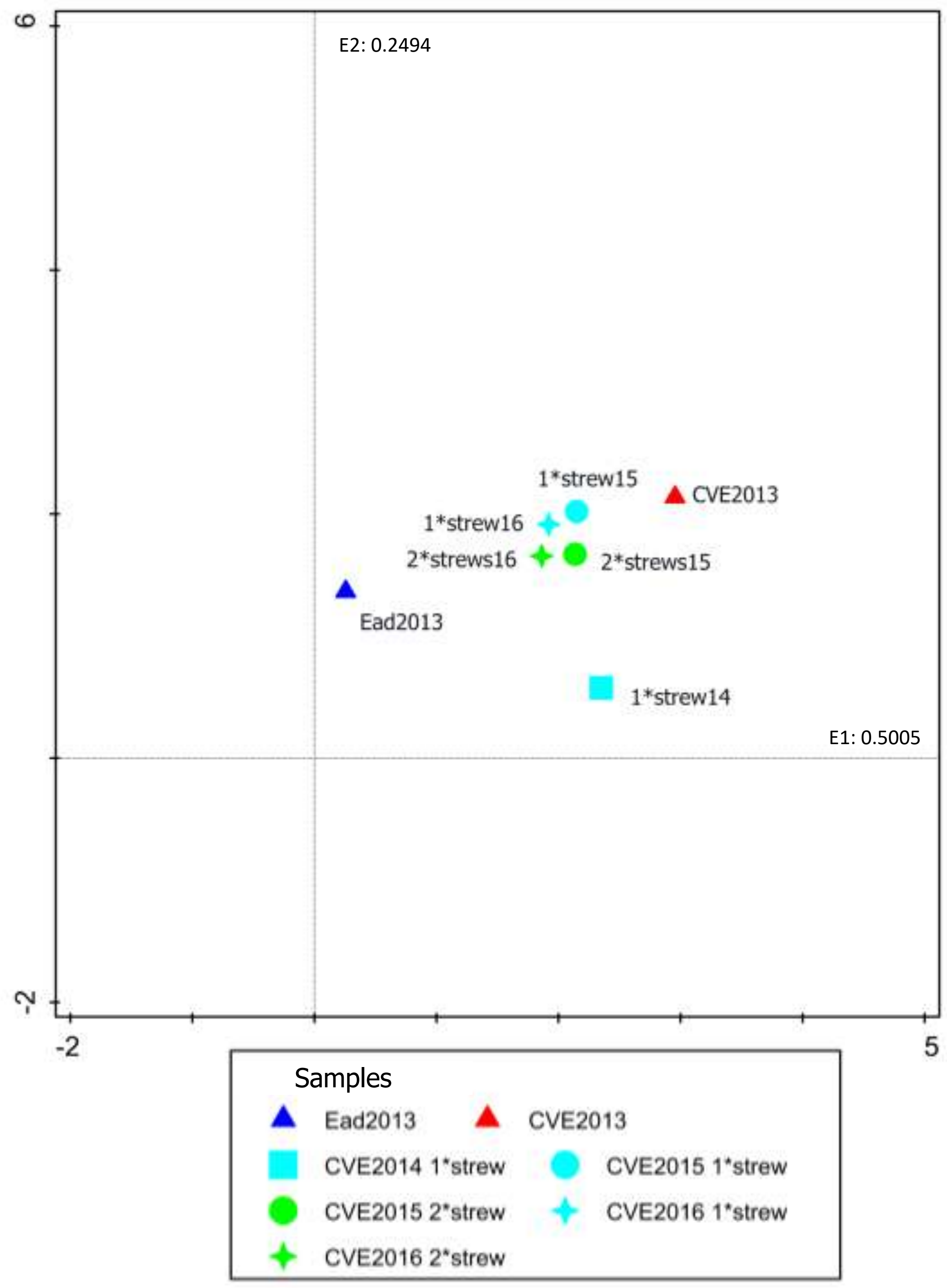
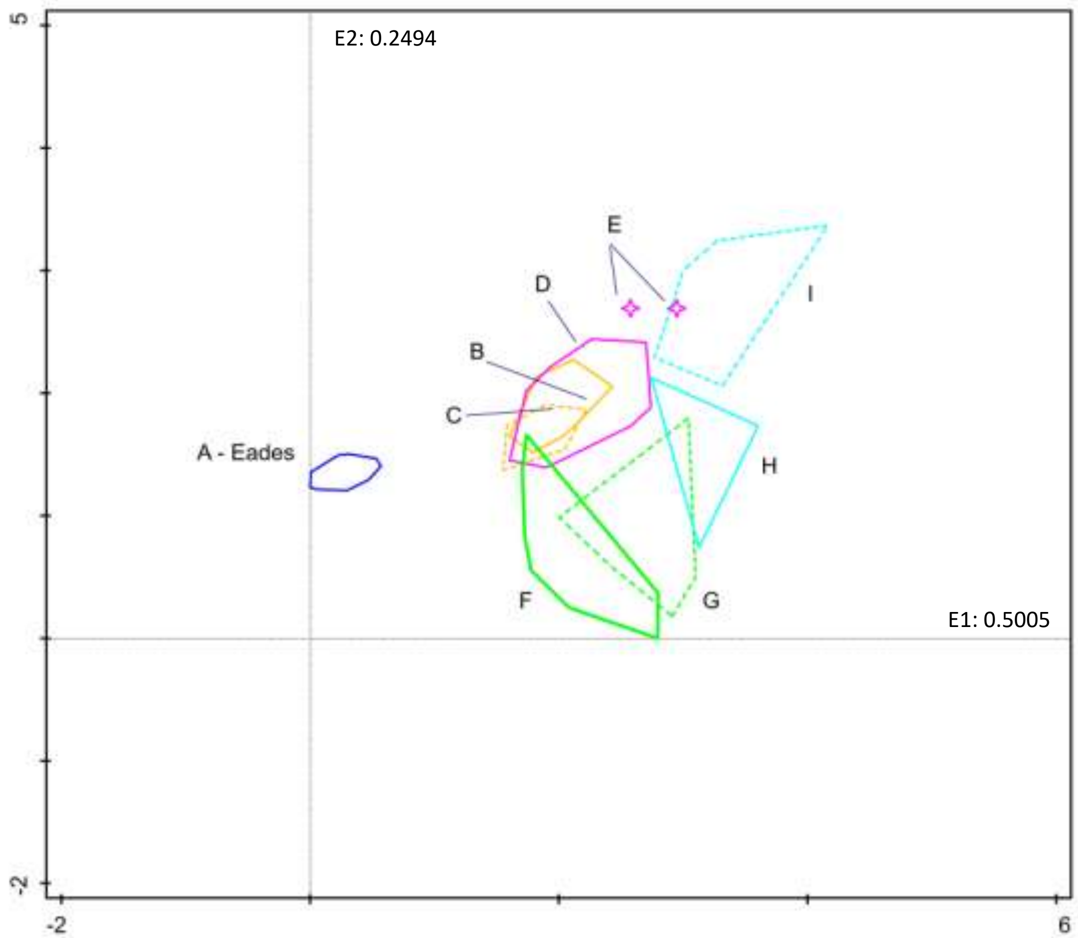



Figure 3.11: Samples plot of the DCA ordination of the Castle Vale and Eades vegetation data, quadrats (samples) coded as environmental variables (nominal treatments) and shown as centroids.




| | | | | | |
|---|---|---|---|---|---|
| **B** | **Group 01000** | CVE2013 – 1<br>CVE2016 – 17/30 2strews<br>24/30 1strew | **C** | **Group 01001** | CVE2016<br>13/30 2strews<br>6/30 1strew |
| **D** | **Group 01010** | CVE2015 – all exc 3 | **E** | **Group 01011** | CVE2013 – 2 |
| **F** | **Group 01100** | CVE2013 – 2<br>CVE2014 – 27/40<br>CVE2015 – 2/30 2strews | **G** | **Group 01101** | CVE2013 – 1<br>CVE2014 – 13/40<br>CVE2015 – 1/30 2strews |
| **H** | **Group 01110** | CVE2013 | **I** | **Group 01111** | CVE2013 |

Figure 3.12: Samples DCA ordination plot with samples coded as their corresponding TWINSPAN groups.

### 3.3.7 Summary of the main results

- 47 species were present at the source but not at the baseline receiver. Of these, 9 were neutral and 2 were undesirable; 18 were on the list of poor performing species from Chapter 1, Table 1.1.
- 21 species were transferred (by 2016) – 16 desirable species and 5 neutral; 8 were on the list of poor performing species from Chapter 1, Table 1.1.
- 20 desirable species did not transfer (12 of which are MG5 species, constancy I and II) and 6 other species (2 of which are MG5 species - constancy I species).
- 16 species increased in the receiver after treatment and were in the source – 14 desirable and 2 neutral species, all MG5 species.
- 1 species (*Scorzoneroides autumnalis*) decreased (was absent after treatment).
- The two-strews treatment had a higher total number and mean number of species per quadrat than the one-strew treatment.
- The first year after treatment had the highest total number of undesirable species and these generally decreased over time.
- The final year of the experiment had the highest number of desirable species and in both years 2-strews had a higher number of desirable species than did 1-strew.
- A factorial repeated measures ANOVA was performed to determine the effects of year and haying frequency levels on the mean percentage cover of the desirable species. It showed that 2016 had significantly higher means

than 2015 and that the two-strew treatment produced markedly higher means than the one-strew treatment, although this difference was not significant. CVE2015 two-strews had the highest diversity and evenness and the lowest dominance – although the most dominant species was different to that of the source meadow.

- The source meadow most closely matched with MG5b. The baseline receiver matched MG1a most closely but by 2016, all groups most closely matched with an MG5 community type (either MG5a or MG5). The overall trend was for a closer match to MG5 over time and for two-strews to have a closer match (to MG5) than one-strew.
- A DCA of the data showed that the two-strews quadrats were more similar to the source meadow than were the one-strew quadrats in each year and that the later year was more similar to the source meadow than the earlier post-treatment years.
- A one-way ANOVA and *post hoc* Tamhane tests showed that the differences between the axis 1 ordination scores of the centroids were statistically significant and that the ordination scores of the centroids for CVE2016 two-strews and CVE2016 one-strew were not significantly different to each other or to the source meadow (Ea2013) on axis 1, but were significantly different to all other groups.

## 3.4 Discussion

### 3.4.1 Community transfer

The results suggest that hay strewing has led to the development of areas at Castle Vale that resemble MG5 grasslands. By 2016, with a mean of 20.62 species per quadrat overall and 23.13 in the two-strews area, the treatment areas approached the species richness of semi-natural meadows, as defined by Grime (1973; species-rich defined as >20 species per $m^2$) and observed by Rodwell in MG5 communities (1992; 23 species per 4 $m^2$, including Bryophytes). Receiver species richness approached, but did not equal source species richness, although it was still some way off equalling the total number of all species and desirable species at Eades Meadow.

The number of transferred species in created meadows has been found to be strongly related to the species-richness of the source site (Kiehl *et al.* 2010; Rayner, 2005). Eades had a high species-richness (a mean of 27.06 species per quadrat), which suggests that it was a good source site and that the number of transferable species should be high. Receiver species-richness was similar to previous studies (e.g. Rayner, 2005; Edwards *et al.,* 2007; Kirkham *et al.,* 2013) and higher than others (e.g. Manchester *et al.,* 1999). The species transfer rate of 58% (species that were transferred plus species that increased) was similar to other studies (e.g. Rayner, 2005, Pywell *et al.,* 2002; Smith *et al.,* 2000).

However, there are still differences between the source and receiver meadows as demonstrated by DCA analysis, by the list of missing species and by the species with the highest total percentage cover (i.e. the most dominant species) being different (*Lotus corniculatus* at the source cf. *Holcus lanatus* at the receiver). There are a number of possible reasons for these differences; for example, created species-rich meadows are known to take time to develop the structure of their semi-natural counterparts (McDonald, 2001; Willems, 2001; Walker *et al.,* 2004; Rayner, 2005; Woodcock *et al.,* 2006) and Castle Vale is still a new meadow. It may, therefore, become more similar to the source meadow over time (assuming appropriate management), as target species become more widely established across the grassland. Specific species may also be over- or under-represented in the sward, for example, *Holcus lanatus*, as mentioned above, was the most dominant species at the receiver. This could be because it produces prolific numbers of seeds (Grime *et al.,* 1988) and the relatively early creation date may have meant that many seeds were present in the hay, especially compared to species that set seed later, leading to a higher proportion of *H. lanatus* in the sward. This species was also observed as a good transferer by Rayner (2005) and Trueman and Millett, (2003). It is an efficient colonist and a generalist species (Grime *et al.,* 1988), which could be additional reasons for its success at Castle Vale. It could also have established from the existing seedbank and/or from the local area. The transfer of individual species is discussed further in Section 3.4.2 below.

After treatment, there was also the appearance of species not generally associated with meadows, such as *Aphanes arvensis, Chamerion angustifolium* and *Geum urbanum*. The majority of these were ruderal species (Grime, 1979; Grime *et al.,* 1988). There are a number of possible reasons for this, including: the herbicide treatment and the consequent high amounts of bare ground that resulted on this site; the open nature of the site and the windy conditions, potentially leading to a high seed rain of these ruderal species and possibly the hotspots of high toxicity of the soil in some areas (Atkins, 2007). This could have lead to the possible failure of germination in some or many species and therefore an increase in bare ground or toxicity that some species can tolerate but others cannot (Antonovics, 1971), although sub-lethal levels may increase diversity (Marrs, 1993; McCrea *et al.,* 2004). Soil toxicity has been found to be negatively correlated with species diversity (Grime, 1973; Rey Benayas and Scheiner, 1993). These non-meadow species tended to be present in 2014, but generally disappeared over time, with appropriate management, as was also found by Pywell *et al.*'s (2003) review of grassland creation and restoration experiments and as experienced by Trueman and Millett (2003). This is illustrated by the TWINSPAN division of group 01 – creating group 011, containing the majority of the quadrats from the baseline and the first year after treatment (2014), plus three from 2015, with *Epilobium ciliatum,* a competitive-ruderal species (Grime *et al.,* 1988) as it's indicator species (Figure 3.8). Conversely, group 010, containing the quadrats from the later years, is characterized by two grass species. This is also illustrated by the centroids ordination plot, where the 2014 centroid is associated with a number of ruderal species. These species are then removed by hay meadow management and the closing of the sward, leading to a lack of regeneration gaps, as observed in the other studies (Trueman and Millett, 2003; Pywell *et al.*, 2003).

### 3.4.2 Species transfer

The percentage frequency present at the source of species that did transfer was generally higher than those that did not (i.e. mean frequency of 64.38% (or 56.44% if species which increased are included[16]) cf. 21.31%). One species of low percentage frequency at the source did transfer, namely *Alopecurus pratensis.* This is a species that has a preference for damp conditions, so it is surprising that it transferred successfully to a receiver where the soils can be 'baked'. However, it is an early flowering species (Grime *et al.,* 1988; Hubbard, 1992) and the meadow was created relatively early in the summer (early July 2013), which may have boosted the number of seeds still connected to the hay, thereby aiding transfer to the new meadow. The conditions could have been wet at germination and the green hay bale may also have been from a damp area at Eades, again boosting the number of seeds. Additionally, although when *A. pratensis* was recorded, in 2015, it was at the same (albeit low) percentage frequency as at the source meadow, it was then not recorded in 2016. This could be due to the low amounts at the receiver, meaning that a quadrat was not located where it

[16] As the meadow was treated with herbicide, all the species present after treatment could be considered to have 'transferred', although there is also the possibility that all of the vegetation was not killed.

was growing, or because it was no longer present at the receiver, due to unsuitable conditions (e.g. soil moisture levels).

Most of the 21 species that transferred, established in the first year after creation, although five (nearly a quarter of the species that transferred) established in the third year, the last of this study. Only four species (*Anthoxanthum odoratum, Arrhenatherum elatius, Crepis biennis* and *Trifolium pratense*) were recorded at similar percentage frequencies in the receiver to that of the source meadow. This under-representation of species that have transferred at the receiver meadow could be due to the young age of the meadow and has been observed in previous studies (as mentioned above, Section 3.4.1). Species with notable low transfer rates include: *Centaurea nigra, Lotus corniculatus* and *Prunella vulgaris*. Possible reasons for this are a cut date that was too early for ripe seed to be present on these species (*C. nigra*, *L. corniculatus* and *P. vulgaris* all set seed from July (Grime *et al.*, 1988)); heavy seed that is lost from the hay *en route* (relevant to *L. corniculatus* (Grime *et al.*, 1988)); or small seed that is lost from the hay *en route*. It is possible that they have transferred, but have not yet been recorded or, for some reason, there may have been little/no viable seed in the creation years or the conditions on the receiver site were not suitable for these species (e.g. competition, amount of disturbance, soil moisture levels).

Three species that did not transfer had percentage frequencies at the source meadow of above 50%, namely: *Carex flacca, Schedonorus pratensis* and

*Galium verum*. *Dactylorhiza fuchsii* also had a relatively high percentage frequency at the source, of 48%. However, orchids are known to take time to appear in created meadows (Trueman and Millet, 2003). Sedges are known to be difficult to germinate, due to their exacting requirements (Schutz, 2000; Holzel and Otte, 2004; Kettenring and Galatowitsch, 2007) and occasional low seed viability (Patzelt *et al.,* 2001). They also set seed early (Holzel and Otte 2003; Donath *et al.,* 2007) and tend to regenerate mainly vegetatively (Grime *et al.,* 1988). However, success has been achieved by some experiments (Trueman and Millett, 2003), although not others (Pakeman *et al.,* 2002; Wagner *et al.,* 2016*)*. *Galium verum* sets seed late (September-November) and has very small seeds (Grime *et al.,* 1988) that could potentially be easily lost from the hay.

Regarding the other species that did not transfer, *Conopodium majus* and *Ophioglossum vulgatum* are early flowering (Grime *et al.,* 1988) and were also only present in small amounts in the source meadow. *Betonica officinalis, Filipendula ulmaria, Succisa pratensis* and *Silaum silaus* are late or at least later flowering (Grime *et al.,* 1988) and *S. pratensis* and *S. silaus* were also only present in small amounts. *S. pratensis* also has a preference for damp conditions (Grime *et al.,* 1988), which may be why it has not established. It may also be present but not yet recorded, as it can take four years to flower (Adams, 1955). *S. silaus* has small seeds, which may be easily lost from the hay.

Four of the species not yet recorded in the experimental plots are orchids, including *D. fuchsii.* As mentioned above, orchids are slow to establish, taking a number of years to appear (Trueman and Millett, 2003), which is a possible reason for their apparent absence in these treatment areas. This could be due to the number of years that orchids can take to flower, and therefore become more conspicuous, for example, *Neottia ovata* flowers when plants are 7-15 years old (Kotilinek *et al.,* 2015). However, orchids do not have endosperm (nutrition) to supply the energy needed for germination, meaning that in the wild they depend entirely on symbiotic mycorrhizae for germination to occur (Rasmussen, 1995; Arditti and Ghani, 2000; Smith and Read, 2008). The absence of these mycorrhizae at the strewing site or in the hay could be another reason for the failure of these species.

Of the remaining species that did not transfer, *Filipendula ulmaria, Galium palustre* and *Ajuga reptans* have a preference for damp conditions (Grime *et al.,* 1988). *Plantago media* has a preference for calcareous soil (Grime *et al.,* 1988) and was only present in the source in small amounts. *Linum catharticum* is low-growing (so may have been missed by the cutting and/or baling equipment) and has small seeds, which may have been lost from the hay. Similarly, *Potentilla reptans* is low growing and was only present in the source in small amounts. *Equisetum arvense, Crataegus monogyna, Hordeum secalinum* and *Phleum pratense* were all present in the source in only small amounts. *E. arvense* is also thought to reproduce mainly vegetatively (Grime *et al.,* 1988) and *C. monogyna* was a sapling, not yet old enough to produce seed. These two species are not desirable and are not target species.

It is not clear why *Allium vineale, Schedonorus pratensis, Medicago lupulina* and *Vicia cracca* did not transfer. Possible explanations could include: successful transfer, but not yet recorded, little/no viable seed in the creation years for some reason or conditions on the receiver site are unsuitable for these species (e.g. competition, amount of disturbance, soil moisture levels). *Vicia cracca* is probably the most surprising of these four species as it is usually easy to transfer (Trueman and Millett, 2003). It is also widely distributed and can become dominant, although it is not a good colonizer, possibly due to its large seed size (Grime *et al.,* 1988).

### 3.4.3 Changes in frequencies of existing species

As mentioned in the results (Section 3.3.1.1), all of the species that were present in the source but were already present in the receiver before treatment, except *Scorzoneroides autumnalis*, subsequently increased substantially[17] in percentage frequency in the receiver meadow, in both the one-strew and two-strew areas. This would be expected, as site conditions must suit these species, if they already existed on the site, and extra seed would therefore be likely to lead to more plants. Conversely, killing off the vegetation could have prevented re-establishment, due to the creation of

[17] As the pre-existing vegetation was killed with glyphosate, 'increased' should mean 're-established at a higher frequency', although there is also the possibility that all of the vegetation was not killed.

bare ground conditions. However, in this case, the dead vegetation was not removed so the ground was not completely bare, providing shelter for germination/ establishment of seedlings. It is also possible that the herbicide did not kill all the existing vegetation or there was re-colonization from local sources, including the seedbank, rather than from the source meadow.

### 3.4.4 Differences between years

In 2014, the first year after creation, the receiver meadow had a high percentage frequency of bare ground (present in 100% of the quadrats) and the vegetation included a high number of undesirable species, which were not recorded in the baseline receiver or the source. This difference between the years and its cause is illustrated by the DCA ordination plots (Figures 3.9-3.11). In 2015 and 2016, there was much less bare ground and the undesirable species are lost or declining, probably due to either the meadow management regime being unsuitable or to the lack of regeneration gaps caused by the sward closing through the establishment and spread of true meadow species along with the lack of grazing (grazing is known to create regeneration gaps; Bullock *et al.,* 2001).

In 2016 the mean and total number of species were both much higher than in any previous year. This pattern is followed by the desirable and neutral species, whereas the number of undesirable species increased in 2014 and decreased to 2016. There appears to be a slow progression towards similarity to the source meadow. The factorial repeated measures ANOVA on the mean percentage cover of the desirable species also showed that there was a significant effect of year, the second year having a higher mean percentage cover of desirable species, than the first year.

**3.4.5 Differences between treatments: one-strew and two-strews**

Nine species were present in two-strews only, two of which were desirable. Twelve species were found in one-strew only, however, the majority were undesirable, non-meadow species such as *Lactuca serriola, Geum urbanum* and *Chamerion angustifolium*. The desirable species *Alopecurus pratensis, Luzula campestris, Leontodon saxatile* and *Lathyrus pratensis* were also found in one-strew only, although the latter two species were not recorded after 2014 (along with many of the non-meadow species). The effect of haying frequency on the mean percentage cover of the desirable species was not significant, although the difference between the two levels of the treatment was noticeable, with two years of haying having a higher mean percentage cover of the desirable species.

Around half of the species were recorded at a substantially higher frequency in the two-strews area compared to the one-strew area in both 2015 and 2016, half or more of these being desirable species. Fourteen species were recorded at significantly higher frequencies in the two-strews area, including 11 desirable species. This suggests there is a benefit of strewing twice and the high number of species recorded at higher frequencies suggests that there is enough of a benefit to recommend this method, although longer term monitoring would be beneficial. However, some species are still missing from the created meadow. Possible solutions to this include: more strews of hay in consecutive years or to wait until vegetation has developed and then carry out another strew. The latter may be required by species that need shelter or other conditions provided by established vegetation before they can establish, rather than the mostly bare ground conditions that exist in a new creation site such as this, as in the normal process of succession (Begon *et al.,* 2006). Conversely, it may be that soil conditions may need to change from high fertility to a lower fertility (the opposite of succession; Smith *et al.,* 2003; Dunn and Tallowin, 2012) to allow these species to establish ('late restoration species'; Dunn and Tallowin, 2012). This may be directly related to soil nutrient levels, or to the effects of competitive plants or to differences in ecosystem functioning; e.g. the differences in functioning between the bacteria-dominated populations of intensively farmed grasslands compared to the predominantly fungal populations of species-rich meadows (Bardgett and McAlister, 1999; Smith *et al.,* 2008; Bardgett *et al.,* 2012; De Vries *et al.,* 2012; Liu *et al*., 2015). There may also be other site conditions that need to improve, change or become more meadow-like.

Studies have also been carried out on facilitator species, with varying results, to investigate if they can aid the establishment of other species, either through the amelioration of bare ground conditions or by altering soil conditions making them more favourable for poorly performing species (Smith *et al.,* 2003; Dunn and Tallowin, 2012; Beaumont *et al.,* 2012). Jefferson (2009) and Smith *et al.* (2003, 2008) suggest that *Rhinanthus* *minor* is one such species. Smith *et al.* (2003, 2008) also suggest that legumes have a beneficial effect on soil microbial communities. For a second strew to be effective in providing facilitator species, it may be that more time is needed between the first and second strewing.

Strewing hay cut at different times of the year may also aid in the transfer of some species (Jones *et al.,* 1995; Walker *et al.,* 2004; Rayner, 2005; Edwards *et al.,* 2007; Natural England, 2010a), although the impacts of this on the source meadow would need to be taken into account, e.g. Smith *et al.,* 1996a,b,c; Smith *et al.,* 2008). However, infrequent late cuts would have been part of normal practise, due to the effects of wet weather, and occasional late cutting may also be important to allowing seed set by late flowering species (Anderson, 1995; Jefferson, 2005).

## 3.5 Conclusions

The species that were missing from Castle Vale compared to Eades Meadow NNR, and therefore the target species for this experiment were: *Alopecurus pratensis, Anthoxanthum odoratum, Arrhenatherum elatius, Avenula pubescens, Briza media, Centaurea nigra, Crepis biennis, Hypochaeris radicata, Lathyrus pratensis, Leontodon hispidus, Leontodon saxatile, Leucanthemum vulgare, Lotus corniculatus, Luzula campestris, Primula veris, Prunella vulgaris, Rhinanthus minor, Rumex acetosa, Tragopogon pratensis, Trifolium pratense, Trisetum flavescens, Ajuga reptans, Allium vineale, Bellis perennis, Betonica officinalis, Carex flacca, Conopodium majus, Crataegus monogyna, Dactylorhiza fuchsii, Equisetum arvense, Filipendula ulmaria,* *Galium palustre, Galium verum, Hordeum secalinum, Linum catharticum, Neottia ovata, Medicago lupis, Anacamptis morio, Ophioglossum vulgatum, Ophrys apifera, Phleum pratense, Plantago media, Potentilla reptans, Schedonorus pratensis, Silaum silaus, Succisa pratensis* and *Vicia cracca.* The first 21 of these species (up to and including *Trisetum flavescens*; 16 desirable, 5 neutral) were transferred to the experimental area, by this experiment and the latter 24 (22 desirable, 4 neutral) were not. Eighteen of these species are on the list of poor performing MG5 species taken from a review of the literature (Chapter 1, Table 1.1). Orchids are also identified in the literature as difficult species to establish in created meadows.

This experiment found that green hay strewing created areas resembling MG5 grasslands after only 2-3 years. As well as the 21 new species introduced by the experiment, 16 species increased substantially in percentage frequency. The species that were not transferred by this experiment overall had a much lower percentage frequency in the source meadow than did the species that did transfer, although some individual species of low frequency did transfer and some individual species of high frequency did not. Desirable species had a significantly higher mean percentage cover in the second year of the experiment, suggesting that the conditions on the site suited these species. Species that were neither in the baseline receiver nor in the source but were in the receiver after treatment were generally undesirable species and these tended to decrease over time.

Desirable species had a higher mean percentage cover in the two-strewed treatment than the one-strewed treatment, but this difference was not statistically significant. The two-strews treatment had a higher mean and total number of species and higher diversity and evenness and lower dominance than the one-strew treatment. The two-strews treatment was also more similar (and more similar earlier) to an MG5 community type than the one-strew treatment. In each year, the two-strews treatment was also more similar to the source meadow quadrats than the one-strew treatment. This suggests that the two-strews treatment had a beneficial effect, so it would be worth repeating the experiment on other sites to fully test the method.

Of the MG5 species identified as performing poorly from a review of the literature (Chapter 1, Table 1.1), one species (*Lotus corniculatus*) was only found in the two-strews treatment (after 2014), one species (*Luzula campestris*) was only found in the one-strew treatment (after 2014), *Dactylis glomerata* was only found in the one-strew treatment in 2015 (and 2014) and in both treatments, in similar amounts in 2016 and *Lathyrus pratensis* was only found in 2014 (and therefore in the one-strew treatment).

There is clearly scope for further work in addition to running a larger experiment with replication and more controlled site conditions, should the funding and a suitable site become available. For example, there are still species missing from the receiver meadow compared to the source meadow, therefore a further strew could take place and the optimum time from creation (and/or stage of development of the receiver meadow) could be investigated. As discussed, the optimum time between the first and second or first and further strews may depend on the stage of development in the receiver; the individual species present or being introduced; other conditions in the meadow (e.g. fertility (McCrea *et al.,* 2004) or soil fungi:bacteria ratios (Smith *et al.,* 2008; Dunn and Tallowin, 2012). Other considerations could be strewing hay cut at different times within a season to aid the establishment of early or late-flowering species. As such hay can be difficult to source, hand collection of these seeds may be a more realistic option. Longer-term monitoring of this experiment should also take place, as long-term monitoring of projects (with appropriate habitat management) is needed to ensure the created habitats become sustainable functioning ecosystems that persist through time (Turnbull *et al.,* 2000; Bakker *et al.,* 1996; Beltman *et al.,* 2007; Ehrlen *et al.,* 2006).

# Chapter 4

# Enhancing an existing created meadow

## 4.1 Introduction

For several reasons, successful created and restored species-rich grasslands may still be lacking in characteristic grassland species – i.e. whichever creation, restoration or enhancement technique is used, not all species establish at the first attempt (Section 1.8). The isolation of species-rich grasslands (Section 1.1.) and the short-lived viability of the majority of grassland species seeds (Grime *et al.,* 1988; Bakker *et al.,* 1996; Mitlacher *et al.*, 2002; Bossuyt and Honnay, 2008), mean that these species are very unlikely to establish or re-establish populations naturally. Target plant species need to be added to such sites (Wells, 1989; Bakker *et al.,* 1996; Coulson *et al.,* 2001; Walker *et al.,* 2004; Holzel, 2012) and therefore the introduction of missing species into the existing vegetation is a possible method to increase the diversity of established, relatively species-rich swards. It should be noted that the NVC is not meant as a description of target communities, habitats of the same (NVC) type show variation across the country (and within regions) and homogeneity of habitats of the same type is not desirable (Rodwell, 1992).

This experiment investigates whether strewing of species-rich green hay onto an existing species-rich receiver, which has been the subject of a previous hay strewing, can increase the number of species present and the frequency and abundance of existing species (particularly those of low frequency and abundance) in the receiver meadow. It also examines which species are difficult to transfer, in order to consider why they are more difficult and what their specific requirements might be.

Aim:

To investigate the effect on species-richness, of strewing green hay from a species-rich source meadow onto a previously species-enriched meadow with existing species richness to introduce more meadow species; i.e. enhancement of an existing created meadow (phased introduction) and increase the evenness of receiver meadow by increasing the frequency of existing meadow species.

Objectives:

To apply a replicated haying treatment (hayed and not-hayed treatment areas) to an enriched meadow with pre-existing diversity.

To compare the vegetation in the receiver meadow (hayed and not-hayed treatment areas) with that of the baseline vegetation and with the source meadow.

## 4.2 Methods

### 4.2.1 Site Descriptions

#### 4.2.1.1 Receiver meadow

Cae Gross (SO314291) is a 1.56 ha hay meadow on Lower Turnant farm, approximately 23 km south-west of Hereford (Figures 4.1, 4.2) and 10 km from the source meadow. The field is generally flat and had previously been strewn with green hay from The Bryn farm, in 2007, in order to improve its impoverished diversity (Tierney pers. comm., 2011; Figure 4.3). Its management is a cut and remove in July with aftermath grazing by sheep or cattle. It is also grazed by sheep or cattle in spring, after which livestock are excluded (i.e. the meadow is 'shut up') (Table 4.1). This management continued throughout the experiment, i.e. for six years (including the walkover survey in 2016).

The original source site (The Bryn) was also a created meadow and was not overly species-rich, therefore this was not suitable to use as a source of hay to increase the diversity of Cae Gross. Pikes Farm was chosen as this was a local, semi-natural, species-rich grassland, designated a SSSI and contained many species that Cae Gross did not. Both the source and the receiver meadows are within the Black Mountains and Golden Valley Natural Character Area and have soils that are neutral to mildly acidic, from Old Red Sandstone (Natural England, 2014).


Figure 4.1: Location of Cae Gross (receiver meadow) and Pikes Farm SSSI (source meadow). Locations are indicated with red markers, the most northerly being Pikes Farm.

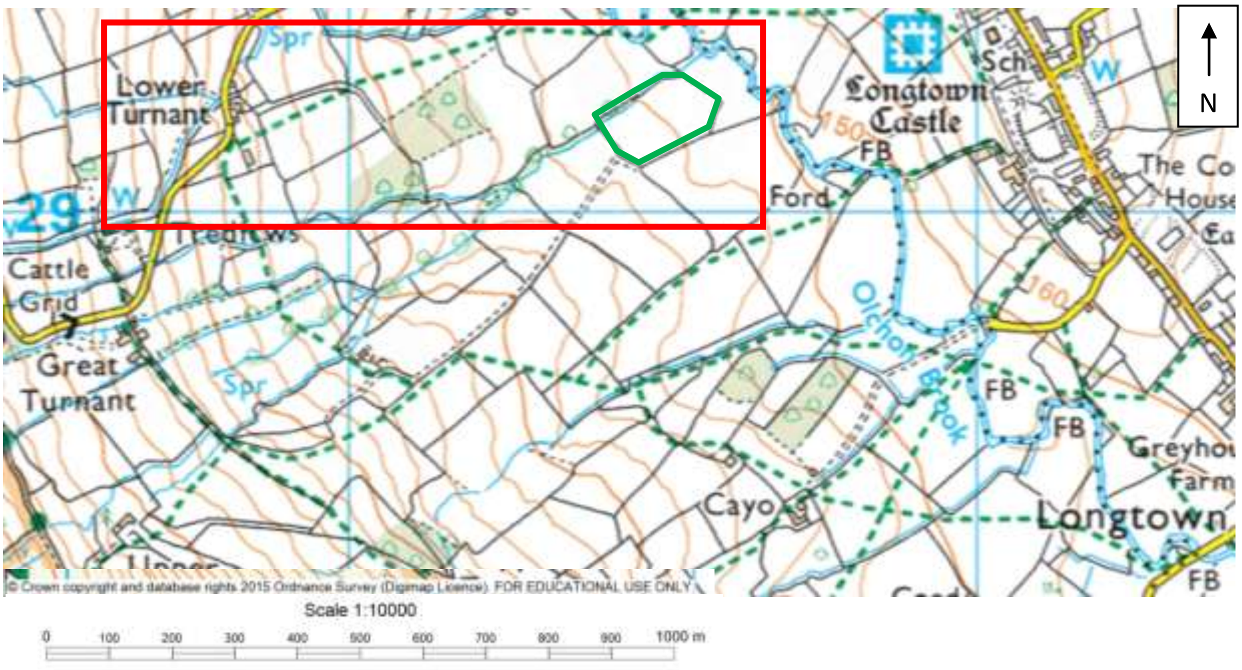



Figure 4.2: Location of Cae Gross (outlined in green) at Lower Turnant (Digimap, 2015).

Figure 4.3: The pre-experiment vegetation in Cae Gross (20.7.2011).

Table 4.1: Site history and management for Cae Gross

| Year | Site history and management |
|---|---|
| **Pre-2011** | Managed as a hay meadow prior to experiment. In 2007, an enrichment project was carried out using hay from The Bryn, funded by Higher Level Stewardship |
| **2011** | Experiment laid down – hay cut and removed, harrowing and strewing of treated areas and controls (no treatment). No autumn grazing |
| **2012** | Spring-grazed by 40 sheep in May for 3 weeks[18] |
| | Hay cut in July |
| | Autumn-grazed by 40 sheep in September and October |
| **2013** | Spring-grazed by 40 sheep in May for 3 weeks |
| | Hay cut in July |
| | Autumn-grazed by 40 sheep in September and October |
| **2014** | Spring-grazed by 40 sheep in May for 3 weeks |
| | Hay cut in July |
| | Autumn-grazed by 40 sheep in September and 20 cattle in October |
| **2015** | Spring-grazed by 20 cattle in May for 3 weeks |
| | Hay cut in July |
| | Autumn-grazed by 20 cattle September-November |

[18] Spring-grazing with sheep and lambs is traditional in this region, especially on meadows close to the farmhouse, where the ewes and lambs can be easily monitored (Tierney pers. comm., 2011; Crofts and Jefferson, 1999).

#### 4.2.1.2 Source Meadow

Pikes Farm Meadows (SO290383) are a group of meadows on the eastern side of the Black Mountains in western Herefordshire (Figure 4.4), at an altitude of around 300m (NE, 1989). The meadows are of the MG5 *Cynosurus cristatus-Centaurea nigra* community, with species such as *Rhinanthus minor, Lathyrus pratensis* and *Ophioglossum vulgatum.* They also contain several species of orchid, including *Platanthera chlorantha* and *Dactylorhiza fuchsii* and are designated a SSSI (Natural England, 1989; Figure 4.5). The soils are neutral to mildly acidic, from Old Red Sandstone (Natural England, 1989, 2014). The meadows are managed by a hay cut and removal of the hay, but this only occurs irregularly (Tierney pers. comm., 2011). The meadow used for the green hay was 1.21 ha and most closely matched the MG5a *Lathyrus pratensis* sub-community.

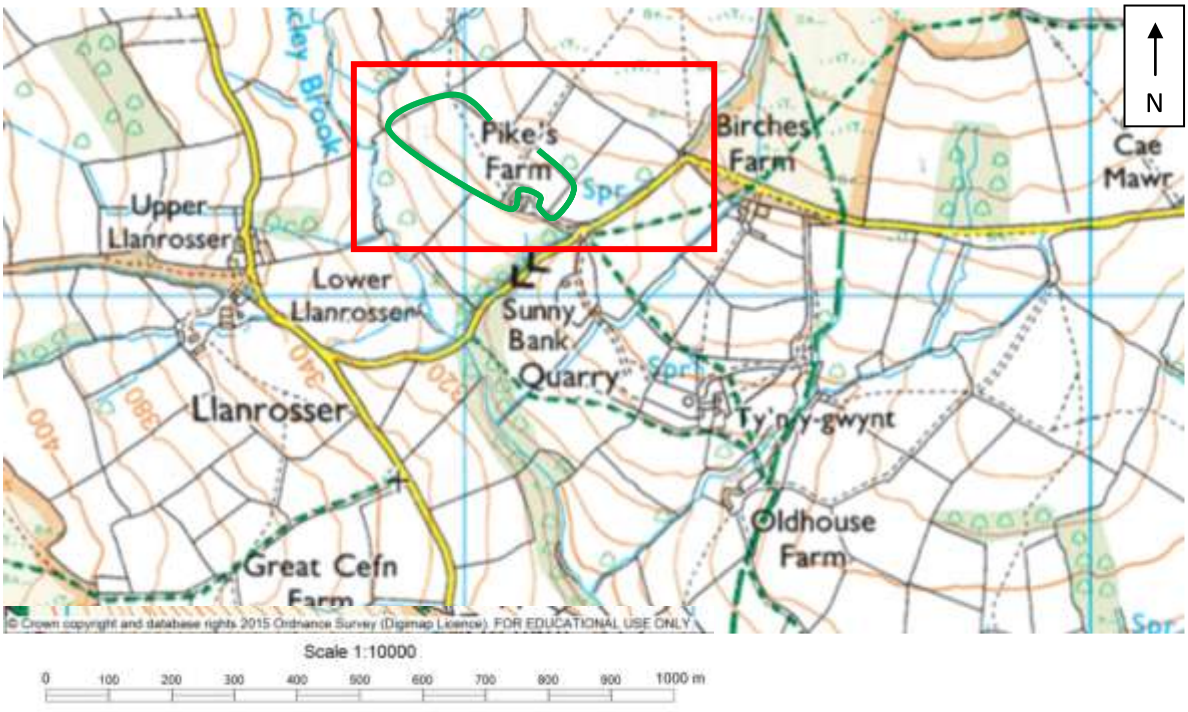



Figure 4.4: Location of Pikes Farm SSSI, source meadow outlined in green (Digimap, 2015).

Figure 4.5: The vegetation in the source meadow at Pikes Farm SSSI (17.6.2011).

#### 4.2.1.3 Comparison of source and receiver sites

Twenty-two species were present at the source but were not present at the receiver, including two *Carex* species, a *Juncus* species, two orchid species and two undesirable species. It is noteworthy that five of these species were only present in small amounts at the source (i.e. ≤2% frequency and low maximum (1%) and mean (0.02%) abundance). These were *Hypericum perforatum, Myosotis arvensis*, *Heracleum sphondylium, Linum catharticum* and *Silene flos-cuculi*. Of the 22 species, one (*Dactylis glomerata*) is an MG5 constancy IV species, one (*Trisetum flavescens*) is a constancy III (41-60% frequency) species, three are constancy II (21-40%) species and five are constancy I (0-20%) species. The remaining 11 species are not MG5 species, but include five desirable ones (*Platanthera chlorantha, Dactylorhiza fuchsii, Myosotis arvensis*, *Linum catharticum, Silene flos-cuculi*) and three neutral species (*Carex leporina, Hypericum perforatum* and *Stellaria graminea*). The remaining four of these 11 species are three undesirable species *(Juncus conglomeratus, Equisetum arvense* and *Rumex obtusifolius*) and one species only identified as *Carex* sp. Species present in CG2011 that were not in Pi2011 include *Taraxacum* spp., *Bromus hordeaceus* and *Bellis perennis*, as well as four undesirable and one neutral species. For more details, see Table 4.4 in the results section.

The species that are present in the source meadow, but absent from the baseline receiver and therefore the target species for this experiment, are: *Betonica officinalis, Carex flacca, Carex* sp., *Conopodium majus*, *Crepis capillaris*, *Dactylis glomerata*, *Dactylorhiza fuchsii*, *Equisteum arvense*, *Heracleum sphondylium*, *Hypericum perforatum, Juncus articulatus, Juncus conglomeratus, Lathyrus pratensis, Leontodon hispidus, Linum catharticum, Myosotis arvensis, Platanthera chlorantha, Rumex obtusifolius, Silene flos-cuculi, Stellaria graminea* and *Trisetum flavescens.* Although these were the main target species, the aim was also to increase the evenness of the receiver meadow by increasing the frequency of existing meadow species. Seven of these species are on the list of poor performing MG5 species from a review of the literature (Chapter 1, Table 1.1). Orchids, of which there are two in the above list, are also identified in the literature as difficult species to establish in created meadows.

### 4.2.2 Experimental design

In July 2011, the receiver meadow was cut and the arisings removed, and treatments of hay and no hay were applied in strips (Figure 4.6). The strips were 50 m long and 5 m wide, except for the double strip which was 10 m wide. The experimental work was carried out by a contractor[19] due to its scale, and strips were the most practical way for this to be done. The hayed strips were power harrowed, the day before the hay was strewn. The machinery was set to create disturbance in the thatch, but minimal disturbance of the soil, i.e. to a maximum depth of about 1 cm. The vegetation cover was approximately 50% after the disturbance had been applied. On 27th July, the source meadow was then cut (at a height of 2-3 cm) and baled and the bales were transported to the receiver meadow, rolled out and spread using a tedder[20] on the same day. Four hayed strips were created and two strips were left without hay to act as controls. The location of the strips was allocated randomly and the application rate was 1:3.

Fifty quadrats were surveyed in both the source and the receiver in July 2011 (pre-treatment), located using random co-ordinates across the whole of each the site (Tables 4.2 and 4.3). In the first four years after treatment,

[19] The owner of The Bryn, Chapter 5 and the contractor for the earlier enrichment work at this site.
[20] Machinery normally used to spread hay to aid drying, photograph in Appendix 2.2.

Fifty-four quadrats were surveyed at the receiver site, with nine quadrats surveyed in each strip, using random numbers to locate quadrats along a transect in the middle of the strip. These were carried out in July 2012 and June 2013-2015. During these surveys any additional species, outside the quadrats, were also recorded as a species list. A walkover survey was carried out on 29th June 2016, walking across as much of the site as possible, in a 'W' pattern, listing all species seen.

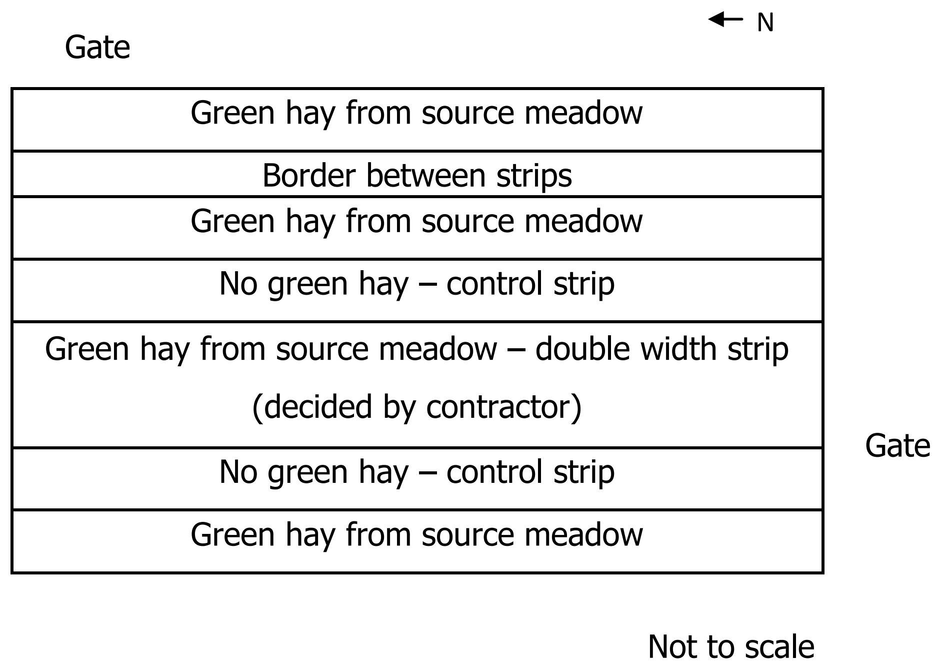



Figure 4.6: Experimental layout at Cae Gross.

Table 4.2: Survey dates for Cae Gross (CG) and its source meadow

| Date | Meadow |
|---|---|
| 5.7.11 | Source meadow, Pikes Farm SSSI |
| 20.7.11 | Receiver, before treatment |
| 19.7.12<br>27.6.13<br>23.6.14<br>25.6.15<br>29.6.16 | Receiver, after treatment |

Table 4.3: Datasets from vegetation surveys

| Datasets from surveys | Number of quadrats |
|---|---|
| Source meadow, Pikes Farm SSSI | 50 |
| Receiver meadow, Cae Gross, Lower Turnant | |
| All quadrats 2011 – before treatment<br>All quadrats – after treatment<br>Hayed<br>Not hayed | 50<br>54<br>36<br>18 |

### 4.2.3 Data preparation and analysis

#### 4.2.3.1 Significance testing of treatment effects

A factorial repeated measures ANOVA was conducted to determine the effects of year (4) and haying (2) levels on the mean percentage cover of the desirable species (following Laerd Statistics (2013)). The source and the baseline were not included in this analysis, the latter because it had not been possible to identify the treatment blocks in advance of the strewing.

#### 4.2.3.2 Comparisons of Canoco PCA centroid scores

One-way ANOVA was used to compare the Canoco PCA centroid axis 1 scores. Treatment blocks were not included as there were none for the source and baseline.

## 4.3 Results

### 4.3.1 Comparison of species in the source and receiver meadows before and after treatment

#### 4.3.1.1 Receiver meadow baseline (CG2011) and after treatment (CG2012-2015)

Ten species, which were not found in the receiver meadow baseline but did occur in the source meadow, appeared in the receiver meadow after treatment (i.e. were transferred; Table 4.4a). These were the one constancy III species, two constancy II species, two constancy I species and six non-MG5 species (nine desirable and one neutral species). Three of the species were the list of poor performing species (Table 1.1, Chapter 1), plus two orchids. The percentage frequencies of these 10 species in the source meadow ranged from 72% (*Leontodon hispidus*) to only being recorded in the walkover (*Linum catharticum*; Table 4.4a), although only *L. catharticum* and *Myosotis arvensis* were <6% frequency. Their mean percentage frequency in the source meadow was 18.89%. Excluding *L. hispidus,* which had a range of abundance in the quadrats from 0% cover to 50% cover and a mean abundance/percentage cover of 14.04%, the species that transferred all had a maximum abundance of ≤5% at the source meadow (Appendix 4.1). Their mean abundance (excluding *L. hispidus*) was ≤0.48%.

Eleven species were found in the source meadow, but not in the receiver before or after treatment (i.e. were not transferred; Table 4.4b). Their frequencies in the source meadow ranged from 26% to only being recorded in the walkover. Their mean percentage frequency in the source meadow was 10.00%. They included two undesirable species and five species that are neither desirable nor undesirable (i.e. neutral (four species), or not identified to species level (*Carex* sp.)), although two of these are MG5 species (constancy IV and I). The maximum abundance for these species was 7%, although five species had a maximum of 1%. The mean percentage cover for these species ranged from 0% (species only seen in the walkover) to 0.46% (Appendix 4.1).

Three other species were found in the receiver meadow after treatment that had not been recorded before, but these were also not in the source meadow (Table 4.4c). One of these was *Ranunculus bulbosus*, an MG5 constancy III species, which increased from a frequency of 2% in 2012 to 19% by 2015. The remaining species are not MG5 species and were only recorded from 2014 and at low frequencies. The new species that were found in the receiver meadow after treatment were not necessarily found in or only in the hayed strips.

Twelve species present in the source and receiver before treatment increased substantially afterwards (Table 4.4d). These species were: *Cynosurus cristatus, Festuca rubra*, *Holcus lanatus, Centaurea nigra,* *Cerastium fontanum*, *Euphrasia* sp.*, Hypochaeris radicata*, *Luzula campestris, Plantago lanceolata, Rhinanthus minor, Rumex acetosa* and *Trifolium dubium*. Their mean percentage frequency in the source meadow was 59.67%.

Nine species were present in the receiver before treatment, but decreased afterwards. These species were: *Agrostis stolonifera*, *Bellis perennis, Cirsium arvense, Poa pratensis*, *Leontodon autumnalis, Lotus corniculatus, Potentilla erecta, Ranunculus repens* and *Trifolium repens*. The last six species were present in the source meadow, the first three were not. Their mean percentage frequency in the source meadow was 34.33%.

Table 4.4: A comparison of the species and their percentage frequencies, recorded in the source meadow (Pi2011) and in the receiver meadow (Cae Gross) each year

w/o indicates species seen on a walkover of the field but not recorded in a quadrat. Text in red highlights differences in presence/absence between meadows or years. D denotes desirable species, U are undesirable and N are species that are neither undesirable nor particularly desirable (neutral) for MG5 hay meadow communities

| | **Pi2011** | **2011** | **2012** | | | **2013** | | | **2014** | | | **2015** | | | **MG5 Const-ancy** | **Desir-ability** |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | **all** | **hay** | **no hay** | **all** | **hay** | **no hay** | **all** | **hay** | **no hay** | **all** | **hay** | **no hay** | | |
| **4.4a Species present in post-treatment receiver but not before treatment and were found in the source (i.e. species that were transferred by the treatment in this experiment)** | | | | | | | | | | | | | | | | |
| *Betonica officinalis* | 8 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | **2** | 3 | 0 | I | D |
| *Carex flacca* | 6 | 0 | **w/o** | **w/o** | 0 | **13** | 8 | 22 | **0** | 0 | 0 | 0 | 0 | 0 | I | D |
| *Dactylorhiza fuchsii* | 16 | 0 | 0 | 0 | 0 | **6** | 0 | 17 | 2 | 0 | 6 | 2 | 3 | 0 | - | D |
| *Lathyrus pratensis* | 10 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | **w/o** | 0 | 0 | 0 | 0 | 0 | II | D |
| *Leontodon hispidus* | 72 | 0 | **20** | 4 | 11 | 35 | 28 | 50 | 43 | 61 | 6 | 41 | 58 | 6 | II | D |
| *Linum catharticum* | w/o | 0 | 0 | 0 | 0 | **4** | 3 | 6 | 4 | 6 | 0 | **0** | 0 | 0 | - | D |
| *Myosotis arvensis* | 2 | 0 | **13** | 0 | 22 | 15 | 0 | 44 | 17 | 3 | 44 | 24 | 11 | 50 | - | D |
| *Platanthera chlorantha* | 24 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | **w/o** | 0 | 0 | - | D |
| *Stellaria graminea* | 6 | 0 | **w/o** | **w/o** | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | - | N |
| *Trisetum flavescens* | 26 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | **w/o** | 0 | 0 | **4** | 3 | 6 | III | D |
| Mean percentage frequency | 18.89 | | | | | | | | | | | | | | | |
| | | | | | | | | | | | | | | | | |
| **4.4b Species which were never found in the receiver meadow, but were in the source** | | | | | | | | | | | | | | | | |
| *Carex* sp. | 2 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | - | - |
| *Conopodium majus* | 46 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | I | D |
| *Crepis capillaris* | 4 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | I | D |

Table 4.4: A comparison of the species and their percentage frequencies, recorded in Pi2011 and in Cae Gross each year, continued

| | Pi2011 | 2011 | 2012 | | | 2013 | | | 2014 | | | 2015 | | | MG5 Const-ancy | Desir-ability |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | all | hay | no hay | all | hay | no hay | all | hay | no hay | all | hay | no hay | | |
| *Dactylis glomerata* | 8 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | IV | N |
| *Equisteum arvense* | 4 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | - | U |
| *Heracleum sphondylium* | w/o | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | II | D |
| *Hypericum perforatum* | 2 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | - | N |
| *Juncus articulatus* | 12 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | I | N |
| *Juncus conglomeratus* | 8 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | - | N |
| *Silene flos-cuculi* | w/o | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | - | D |
| *Rumex obtusifolius* | 4 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | - | U |
| Mean percentage frequency | 10.00 | | | | | | | | | | | | | | | |
| | | | | | | | | | | | | | | | | |
| **4.4c Species present in post-treatment receiver that were not recorded before treatment nor in the source** | | | | | | | | | | | | | | | | |
| *Galium palustre* | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | **w/o** | 0 | 0 | 0 | 0 | 0 | - | D |
| *Ranunculus bulbosus* | 0 | 0 | **2** | 4 | 0 | 13 | 19 | 0 | 11 | 11 | 11 | 19 | 19 | 17 | III | D |
| *Tragopogon pratensis* | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | **w/o** | 0 | 0 | **2** | 3 | 0 | - | D |
| | | | | | | | | | | | | | | | | |
| **4.4d Species that increased substantially after treatment** | | | | | | | | | | | | | | | | |
| *Centaurea nigra* | 96 | 8 | 20 | 11 | 44 | 9 | 8 | 11 | 46 | 44 | 50 | 44 | 28 | 78 | IV | D |
| *Cerastium fontanum* | 56 | 26 | 65 | 78 | 44 | 39 | 33 | 50 | 50 | 44 | 61 | 57 | 64 | 44 | II | D |
| *Cynosurus cristatus* | 18 | 84 | 80 | 96 | 44 | 72 | 81 | 56 | 96 | 94 | 100 | 100 | 100 | 100 | V | D |
| *Euphrasia* sp. | 96 | 8 | 59 | 89 | 17 | 69 | 89 | 28 | 72 | 78 | 61 | 39 | 44 | 28 | - | D |
| *Festuca rubra* agg. | 48 | 86 | 96 | 100 | 100 | 89 | 97 | 72 | 91 | 92 | 89 | 100 | 100 | 100 | V | D |
| *Holcus lanatus* | 88 | 88 | 74 | 89 | 39 | 83 | 92 | 67 | 100 | 100 | 100 | 100 | 100 | 100 | IV | D |
| *Hypochaeris radicata* | 58 | 78 | 89 | 100 | 100 | 93 | 89 | 100 | 81 | 75 | 94 | 89 | 83 | 100 | III | D |
| *Luzula campestris* | 12 | 24 | 74 | 93 | 33 | 43 | 56 | 17 | 15 | 19 | 6 | 52 | 67 | 22 | III | D |

Table 4.4: A comparison of the species and their percentage frequencies, recorded in Pi2011 and in Cae Gross each year, continued

| | Pi2011 | 2011 | 2012 | | | 2013 | | | 2014 | | | 2015 | | | MG5 Const-ancy | Desir-ability |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | all | hay | no hay | all | hay | no hay | all | hay | no hay | all | hay | no hay | | |
| *Plantago lanceolata* | 80 | 36 | 61 | 48 | 100 | 91 | 86 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | V | D |
| *Rhinanthus minor* | 98 | 70 | 100 | 100 | 100 | 98 | 97 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | II | D |
| *Rumex acetosa* | 40 | 16 | 24 | 22 | 0 | 35 | 44 | 17 | 33 | 42 | 17 | 35 | 36 | 33 | III | D |
| *Trifolium dubium* | 26 | 36 | 43 | 15 | 72 | 37 | 28 | 56 | 54 | 47 | 67 | 76 | 72 | 83 | II | D |
| Mean percentage frequency | 59.67 | | | | | | | | | | | | | | | |
| | | | | | | | | | | | | | | | | |
| **4.4e Species that decreased substantially after treatment** | | | | | | | | | | | | | | | | |
| *Agrostis stolonifera* | **0** | 10 | 11 | 22 | 0 | 6 | 6 | 6 | 0 | 0 | 0 | 0 | 0 | 0 | I | U |
| *Bellis perennis* | **0** | 6 | **0** | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | II | D |
| *Cirsium arvense* | **0** | 44 | 13 | 11 | 11 | 6 | 8 | 0 | 9 | 11 | 6 | 4 | 6 | 0 | II | U |
| *Scorzoneroides autumnalis* | 4 | 100 | 50 | 74 | 33 | 39 | 19 | 78 | 52 | 53 | 50 | 70 | 75 | 61 | III | D |
| *Lotus corniculatus* | 96 | 98 | 74 | 85 | 72 | 65 | 58 | 78 | 85 | 89 | 78 | 87 | 83 | 94 | V | D |
| *Poa pratensis* | 14 | 76 | 15 | 22 | 0 | 7 | 11 | 0 | 17 | 17 | 17 | 52 | 44 | 67 | II | D |
| *Potentilla erecta* | 40 | 96 | 98 | 100 | 94 | 81 | 83 | 78 | 81 | 81 | 83 | 87 | 86 | 89 | I | D |
| *Ranunculus repens* | 4 | 44 | 30 | 37 | 0 | 30 | 36 | 17 | 24 | 22 | 28 | 22 | 19 | 28 | I | U |
| *Trifolium repens* | 48 | 100 | 19 | 19 | 6 | 6 | 8 | 0 | 30 | 33 | 22 | 61 | 78 | 28 | IV | D |
| Mean percentage frequency | 34.33 | | | | | | | | | | | | | | | |
| | | | | | | | | | | | | | | | | |
| **4.4f Species which did not change in frequency substantially** | | | | | | | | | | | | | | | | |
| *Achillea millefolium* | 2 | 28 | 37 | 56 | 28 | 24 | 25 | 22 | 35 | 47 | 11 | 33 | 36 | 28 | III | D |
| *Agrostis capillaris* | 100 | 100 | 100 | 100 | 100 | 94 | 100 | 83 | 93 | 97 | 83 | 96 | 94 | 100 | IV | D |
| *Anthoxanthum odoratum* | 98 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | IV | D |
| *Bromus hordeaceus* | **0** | 8 | **0** | 0 | 0 | 2 | 0 | 6 | 2 | 3 | 0 | 2 | 3 | 0 | I | N |
| *Juncus effusus* | **0** | 28 | 11 | 15 | 6 | 30 | 28 | 33 | 6 | 6 | 6 | 6 | 6 | 6 | I | N |

Table 4.4: A comparison of the species and their percentage frequencies, recorded in Pi2011 and in Cae Gross each year, continued

| | Pi2011 | 2011 | 2012 | | | 2013 | | | 2014 | | | 2015 | | | MG5 Const-ancy | Desir-ability |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | all | hay | no hay | all | hay | no hay | all | hay | no hay | all | hay | no hay | | |
| *Leucanthemum vulgare* | w/o | 4 | 4 | 7 | 0 | 2 | 0 | 6 | 2 | 3 | 0 | 4 | 3 | 6 | II | D |
| *Lolium perenne* | **0** | 6 | 26 | 33 | 0 | 7 | 8 | 6 | 4 | 3 | 6 | 9 | 11 | 6 | III | D |
| *Nardus stricta* | **0** | 4 | 2 | 0 | 0 | 2 | 0 | 6 | **0** | 0 | 0 | 0 | 0 | 0 | - | N |
| *Prunella vulgaris* | 2 | 64 | 89 | 89 | 94 | 46 | 36 | 67 | 48 | 50 | 44 | 63 | 56 | 78 | III | D |
| *Quercus robur* seedling | **0** | 2 | **0** | 0 | 0 | 0 | 0 | 0 | **2** | 3 | 0 | 0 | 0 | 0 | - | U |
| *Ranunculus acris* | 70 | 92 | 98 | 100 | 100 | 93 | 89 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | III | D |
| *Taraxacum* spp. | **0** | 8 | 11 | 19 | 0 | 17 | 25 | 0 | 11 | 14 | 6 | 13 | 14 | 11 | III | D |
| *Trifolium pratense* | 98 | 96 | 98 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 96 | 94 | 100 | IV | D |
| Mean percentage frequency | 61.67 | | | | | | | | | | | | | | | |

#### 4.3.1.2 Significance testing of treatment effects

The factorial repeated measures ANOVA conducted to determine the effects of year (4) and haying (2) levels on the mean percentage cover of the desirable species (Laerd Statistics, 2013) showed that the interaction between haying and year was not significant [$F(2.918,99.223) = 1.515$, $p = 0.216$, $\varepsilon = 0.973$ (Huynh-Feldt)]. The mean percentage cover of the desirable species increased over time (for both treatments), but the main effect of year was not significant. There was a statistically significant main effect of haying [$F(1,34) = 4.786$, $p = 0.036$], the hayed treatment (0.49, 95% CI, 0.18 to 1.32) having a higher mean percentage cover of desirable species than the not-hayed treatment (0.29, 95% CI, 0.10 to 0.85) for all years. The mean values are low due to a high number of zeros in the data.

### 4.3.2 Comparisons of total number of species per site and species-richness for each quadrat

Of all the groups of quadrats (source/receiver meadow, years and treatments; i.e. datasets), Pikes Farm had the highest total number of species, but not the highest mean number of species per quadrat (Tables 4.5 and 4.6). The receiver meadow hayed quadrats in 2015 had the highest mean and total number of species of all the hayed quadrats. The receiver meadow not-hayed quadrats in 2015 also had the highest mean and total number of species of all the not-hayed quadrats. The combined quadrats for the receiver meadow in 2015 (CG2015all) had the second highest total number of species (37 to 39 in CG2013all). Excepting 2013 (where the mean is only 0.03 lower), the hayed group had a higher mean and total number of species than the not-hayed group in every year. The mean and total number of species for both hayed and not-hayed increased from 2012 to 2015. The mean and total number of desirable species follows the same pattern as the overall number and mean number of species, described above.

Table 4.5: Mean number of species per quadrat for Cae Gross and Pikes

| | **Year of survey** | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | **2011** | | **2012** | | **2013** | | **2014** | | **2015** | |
| | Mean | s.e | Mean | s.e | Mean | s.e | Mean | s.e | Mean | s.e |
| Source meadow(Pi) | 15.5 | 0.3 | N/A | N/A | N/A | N/A | N/A | N/A | N/A | N/A |
| Receiver(CG): | | | | | | | | | | |
| All quadrats | 16.74 | 0.32 | 17.17 | 0.35 | 16.04 | 0.29 | 17.15 | 0.28 | 18.91 | 0.29 |
| CG hayed | N/A | N/A | 18.28 | 0.35 | 16.03 | 0.37 | 17.47 | 0.39 | 19.03 | 0.39 |
| CG not-hayed | N/A | N/A | 14.94 | 0.42 | 16.06 | 0.47 | 16.50 | 0.32 | 18.67 | 0.40 |
| | | | | | | | | | | |
| **Desirable species only** | | | | | | | | | | |
| Source meadow(Pi) | 15.02 | 0.30 | N/A | N/A | N/A | N/A | N/A | N/A | N/A | N/A |
| Receiver(CG): | | | | | | | | | | |
| All quadrats | 15.34 | 0.28 | 16.39 | 0.30 | 15.07 | 0.25 | 16.72 | 0.31 | 18.56 | 0.26 |
| CG hayed | N/A | N/A | 17.00 | 0.31 | 15.22 | 0.34 | 17.03 | 0.43 | 18.67 | 0.35 |
| CG not-hayed | N/A | N/A | 14.56 | 0.35 | 15.28 | 0.38 | 16.11 | 0.37 | 18.33 | 0.38 |

Table 4.6: Total number of species per meadow for Cae Gross and Pikes

| **Meadow/treatment** | **Year of survey** | | | | |
|---|---|---|---|---|---|
| | **2011** | **2012** | **2013** | **2014** | **2015** |
| Source meadow(Pi) | 42 | N/A | N/A | N/A | N/A |
| Receiver(CG): | | | | | |
| All quadrats | 34 | 35 | 39 | 36 | 37 |
| CG hayed | N/A | 33 | 34 | 35 | 37 |
| CG not-hayed | N/A | 26 | 34 | 32 | 35 |
| | | | | | |
| **Desirable species only** | | | | | |
| Source meadow(Pi) | 33 | N/A | N/A | N/A | N/A |
| Receiver(CG): | | | | | |
| All quadrats | 27 | 30 | 32 | 31 | 32 |
| CG hayed | N/A | 28 | 29 | 31 | 32 |
| CG not-hayed | N/A | 23 | 28 | 29 | 30 |

### 4.3.3 Species diversity and similarity measures

From the calculations of the measures relating to Cae Gross and its source meadow (Table 4.7), the receiver meadow hayed quadrats in 2015 (CG2015 hayed) had the highest diversity of all groups in all years, the equal second highest evenness (with the receiver meadow hayed quadrats in 2014 (CG2014 hayed) and the equal lowest dominance (with CG2015 all; *Festuca rubra* being the most dominant species). The similarity to the source meadow using the Czekanowski coefficient varied from year to year (Figure 4.7): in 2012 and 2015, the hayed group was the most similar to the source.

Table 4.7: Species diversity measures for Cae Gross and Pikes Farm SSSI

| | **Simpson's Index** | **Simpson's Measure of Evenness** | **Berger-Parker** | **Species with highest total % cover** |
|---|---|---|---|---|
| | | | | |
| Source meadow (Pikes Farm 2011) | 2.24 | 0.22 | 0.17 | *Ant odo* |
| Receiver meadow (Cae Gross 2011) | 2.40 | 0.32 | 0.20 | *Lot cor* |
| | | | | |
| **Receiver meadow:** | | | | |
| 2012, all quadrats | 2.29 | 0.28 | 0.18 | *Rhi min* |
| 2012 hayed | 2.22 | 0.26 | 0.20 | *Agr cap* |
| 2012 not hayed | 1.95 | 0.27 | 0.27 | *Rhi min* |
| | | | | |
| 2013, all quadrats | 2.08 | 0.21 | 0.25 | *Rhi min* |
| 2013, hayed | 1.81 | 0.18 | 0.35 | *Rhi min* |
| 2013, not hayed | 1.91 | 0.20 | 0.28 | *Tri pra* |
| | | | | |
| 2014, all quadrats | 2.35 | 0.29 | 0.20 | *Tri pra* |
| 2014, hayed | 2.38 | 0.31 | 0.17 | *Tri pra* |
| 2014, not hayed | 2.17 | 0.27 | 0.26 | *Tri pra* |
| | | | | |
| 2015, all quadrats | 2.40 | 0.30 | 0.13 | *Tri pra* |
| 2015, hayed | 2.45 | 0.31 | 0.13 | *Fes rub* |
| 2015, not hayed | 2.16 | 0.27 | 0.19 | *Cyn cri* |

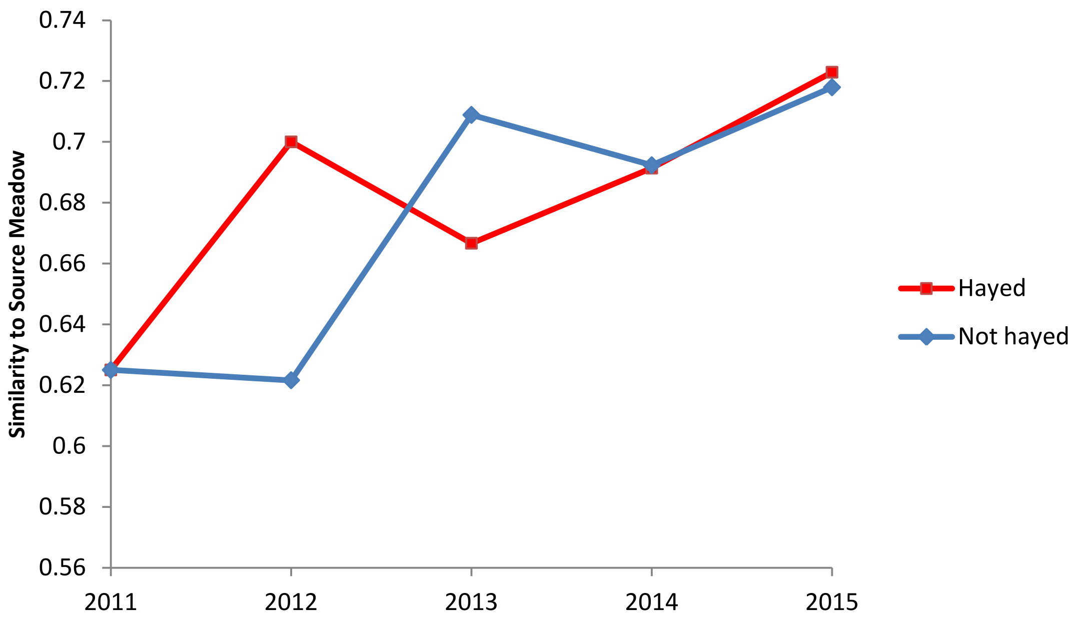



Figure 4.7: Comparison of source and receiver meadow treatments using the Czekanowski coefficient.

### 4.3.4 Comparison with NVC communities

The source meadow (in 2011) most closely matched the MG5a *Lathyrus pratensis* sub-community (Table 4.8). The baseline receiver meadow matched MG6b most closely, whereas all the post-treatment groups (including the not-hayed groups) most closely matched an MG5 community (i.e. MG5, MG5a *Lathyrus pratensis* sub-community, MG5b *Galium verum* sub-community or MG5c *Danthonia decumbens* sub-community). However, the baseline receiver had a closer match to MG5c than the receiver in 2012 and 2013 (Table 4.8). The overall trend was for a closer similarity with MG5 over time and for the hayed group to have the closest match to MG5 of the groups in the year, except in 2015 when the all quadrats group was the most similar to an MG5 community.

Table 4.8: Highest three coefficients of similarity to NVC sub-communities, from MAVIS

| **Source meadow Pikes Farm 2011, all** | **Receiver meadow CG2011, all** | |
|---|---|---|
| MG5a 61.35 | MG6b 67.74 | |
| MG5c 60.81 | MG5c 66.26 | |
| MG5 60.36 | MG5a 64.21 | |
| **Receiver meadow:** | | |
| **2012, all quadrats** | **2012 hayed** | **2012 not-hayed** |
| MG5c 65.71 | MG5c 67.61 | MG5c 59.11 |
| MG5a 64.24 | MG5a 64.57 | MG5a 58.74 |
| MG5 64.18 | MG5 64.52 | MG5 57.90 |
| **2013, all quadrats** | **2013 hayed** | **2013 not-hayed** |
| MG5c 64.62 | MG5c 64.86 | MG5c 59.76 |
| MG5a 63.96 | MG5a 64.20 | MG5a 57.68 |
| MG5 63.89 | MG5 64.14 | MG5 57.53 |
| **2014, all quadrats** | **2014 hayed** | **2014 not-hayed** |
| MG5c 66.75 | MG5c 68.10 | MG5a 63.27 |
| MG5a 66.21 | MG5a 67.64 | MG5c 63.15 |
| MG5 65.30 | MG5 66.75 | MG5 62.31 |
| **2015, all quadrats** | **2015 hayed** | **2015 not-hayed** |
| MG5c 70.67 | MG5c 69.37 | MG5c 67.80 |
| MG5a 68.45 | MG5 67.90 | MG5a 67.06 |
| MG5 68.45 | MG5a 67.08 | MG5 66.18 |

## 4.3.5 Principal Component Analysis (PCA)

### 4.3.5.1 All years and treatments ordination plots

A PCA ordination plot of the Cae Gross source and receiver samples (quadrats; Figure 4.8) shows that the source and baseline receiver are close together on axis 1 and axis 2. The envelopes showing the spread of the quadrats of the source and receiver overlap, although the quadrats of the source are less widely spread, on axis 1. In 2012 and 2013, both the hayed and not-hayed quadrats are further away from the source than the baseline, showing no overlap with the source.  The hayed quadrats have higher ordination scores on axis 1 than the source and baseline receiver, although the hayed quadrats have a small overlap with those of the baseline. The not-hayed quadrats have higher ordination scores on axes 1 and 2 and have no overlap with the baseline.

The corresponding species plot (Figure. 4.9) suggests that the 2013 hayed group of quadrats is associated with *R. minor ,* as it is located at a similar position on the plot (Figures 4.8 and 4.9). The not-hayed group appears to be associated with *T. pratense*. These are also the species with the highest total percentage cover in these groups (4.3.3). The species in each of the various colour-coded categories (Figure 4.9), e.g. species that transferred or those that appeared to increase substantially, are fairly broadly spread across the plot, on both axes.

From 2014, the quadrats have lower ordination scores on both axes, although they are close to those of the baseline (Figure 4.8). The hayed quadrats are slightly nearer to the source and baseline than the not-hayed quadrats; in 2015, both the hayed and not-hayed quadrat groups overlap with both the baseline and the source. A plot of the centroids of the treatment groups (Figure 4.10) more clearly demonstrates that the hayed groups are more similar to the baseline and the source than their not-hayed counterparts and also how the sample plots differ from year to year.

The quadrats in the post-treatment groups are more widely spread (i.e. their envelopes cover a wider area on the plot) than the source and the baseline receiver quadrats; this is especially true in 2013. There is overlap between the hayed and not-hayed quadrats in every year except 2013 (Figure 4.8).

#### 4.3.5.2 Individual years' ordination biplots

Samples and species biplots of the individual years (Figure 4.11) show that, in 2012 the hayed and not-hayed quadrats are mostly in separate areas of the plot, with the hayed quadrats associated with a higher number of species and species with longer arrows[21] of *L. corniculatus, L. campestris, C. cristatus* and *A. capillaris*. The not-hayed quadrats are associated with *R. minor, H. radicata, P. lanceolata* and *R. acris*. In 2013, there is no overlap of the treatment groups, but in 2014 and 2015 there are large overlaps. In all years the hayed quadrats are more widely spread, at least on axis 2 than the not-hayed quadrats. The species associations are similar in all years, except that in 2013 and 2014 *R. minor* becomes more associated with the hayed quadrats and by 2015 *C. cristatus* becomes more associated with the not-hayed quadrats.

[21] Species that are more strongly associated with the variation explained by the plot.

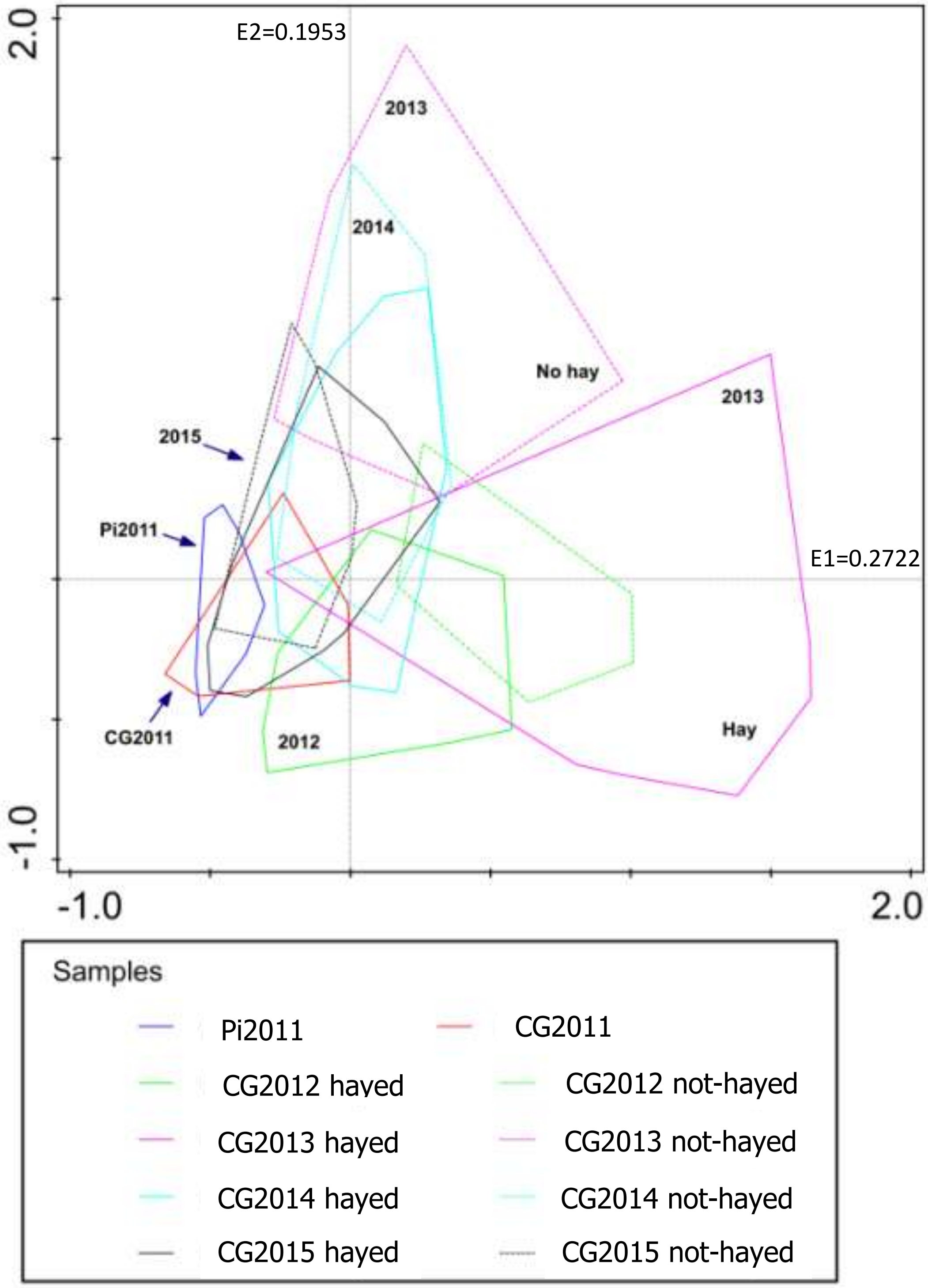
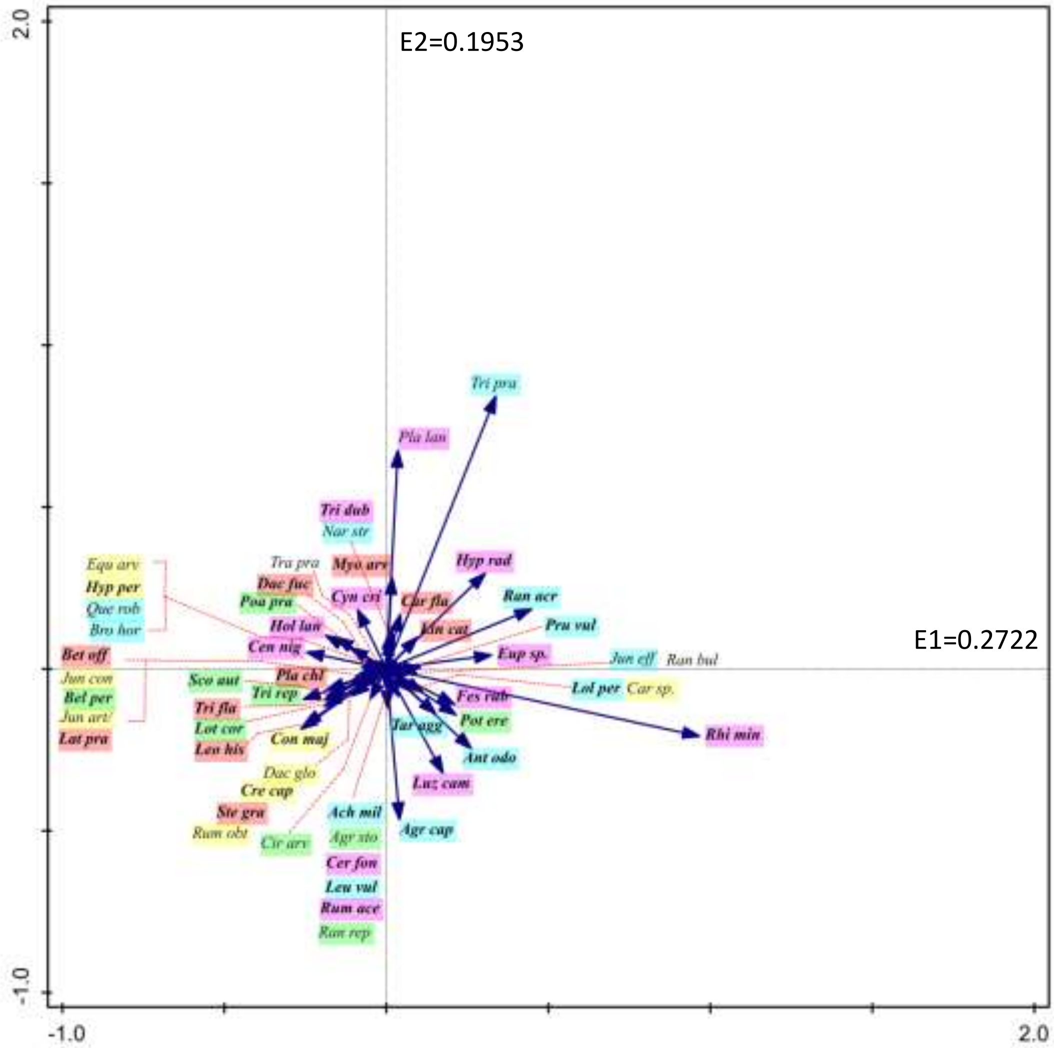



Figure 4.8: PCA samples ordination plot of Cae Gross source and receiver samples.

Samples shown as envelopes. E1 is the eigenvalue for axis 1 and E2 the eigenvalue for axis 2. Eigenvalues measure the extent of variation among the samples that the axis explains (ter Braak and Smilauer, 2012).




Species which were found in the receiver post treatment but not before treatment and were found in the source (i.e. transferred)

Species that appeared to increase substantially post-treatment

Species that appeared to decrease substantially post-treatment

Species that appeared not to change post-treatment

Species that did not transfer Species in bold are desirable species;

Species not colour coded are species that were not in the receiver before treatment but were not in the source – of these only *Ran bul* is desirable.

Figure 4.9: PCA species ordination plot of Cae Gross source and receiver samples.

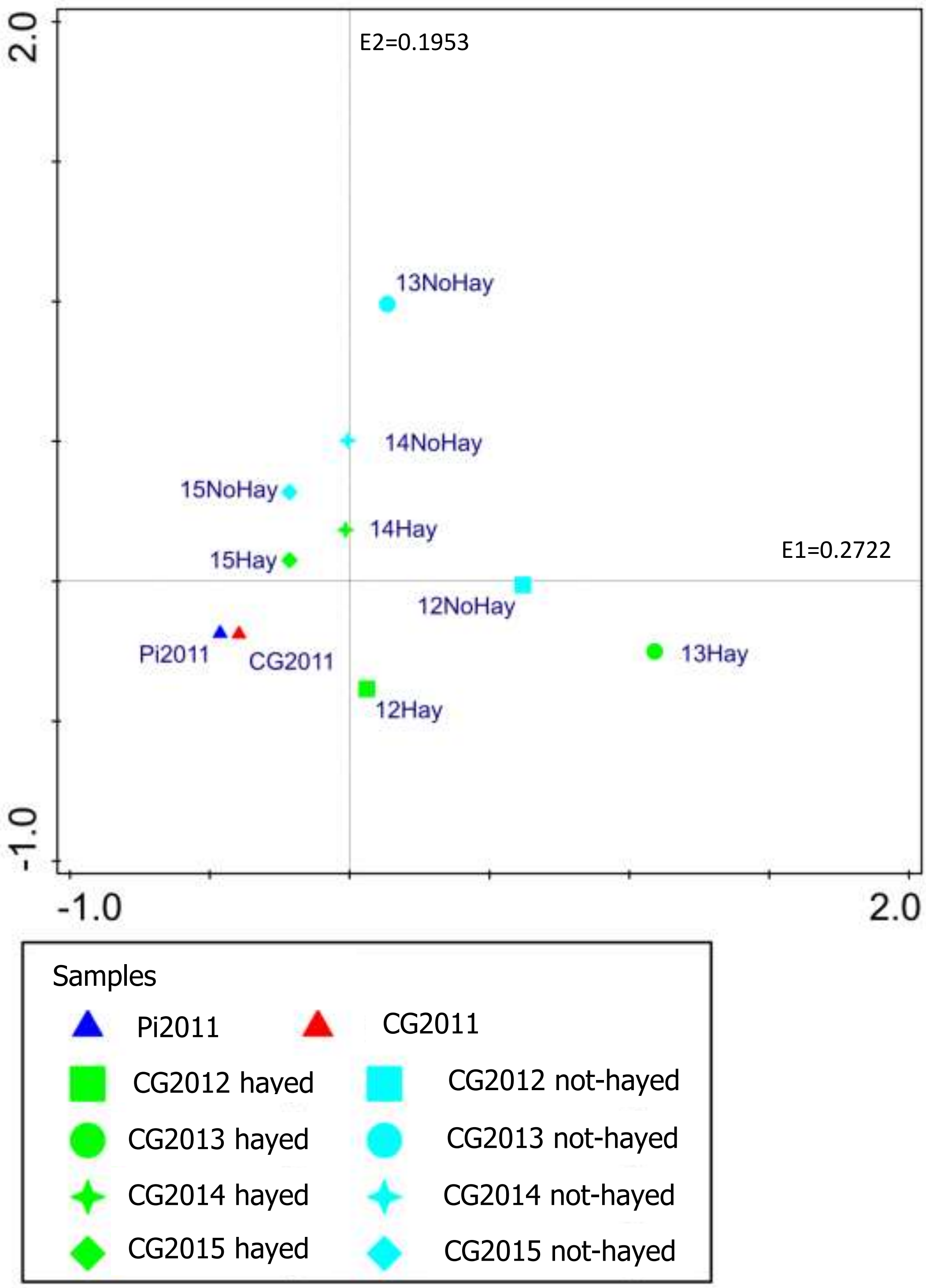
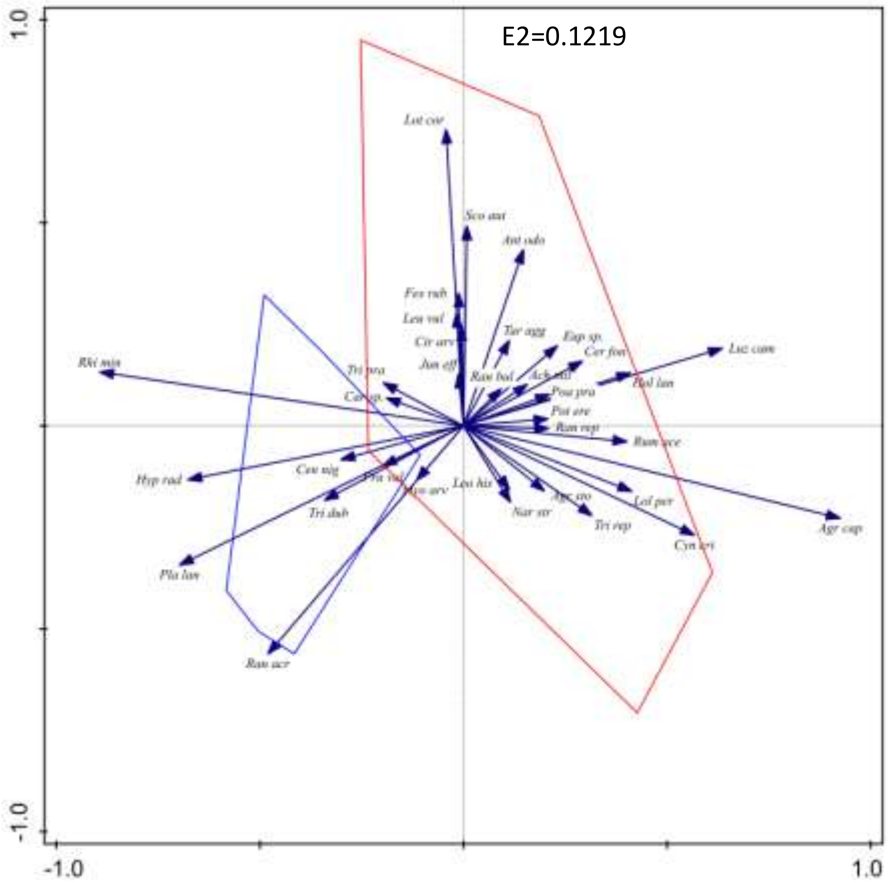
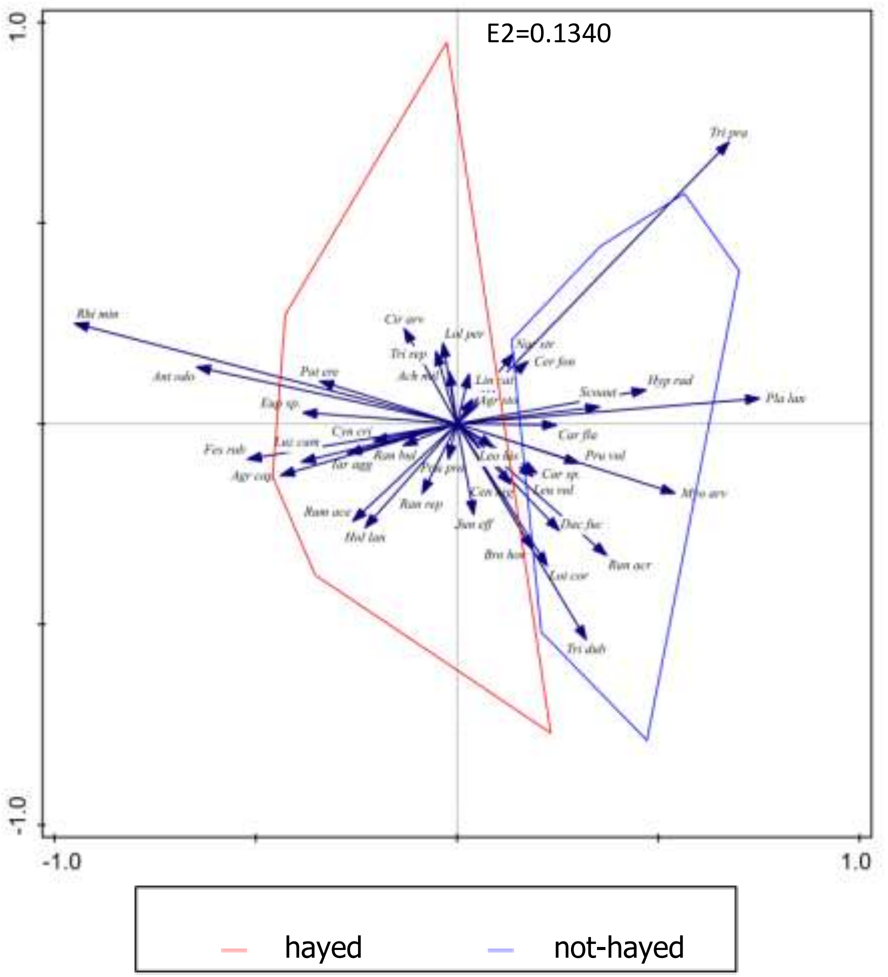



Figure 4.10: PCA samples ordination plot of Cae Gross source and receiver with samples shown as centroids.



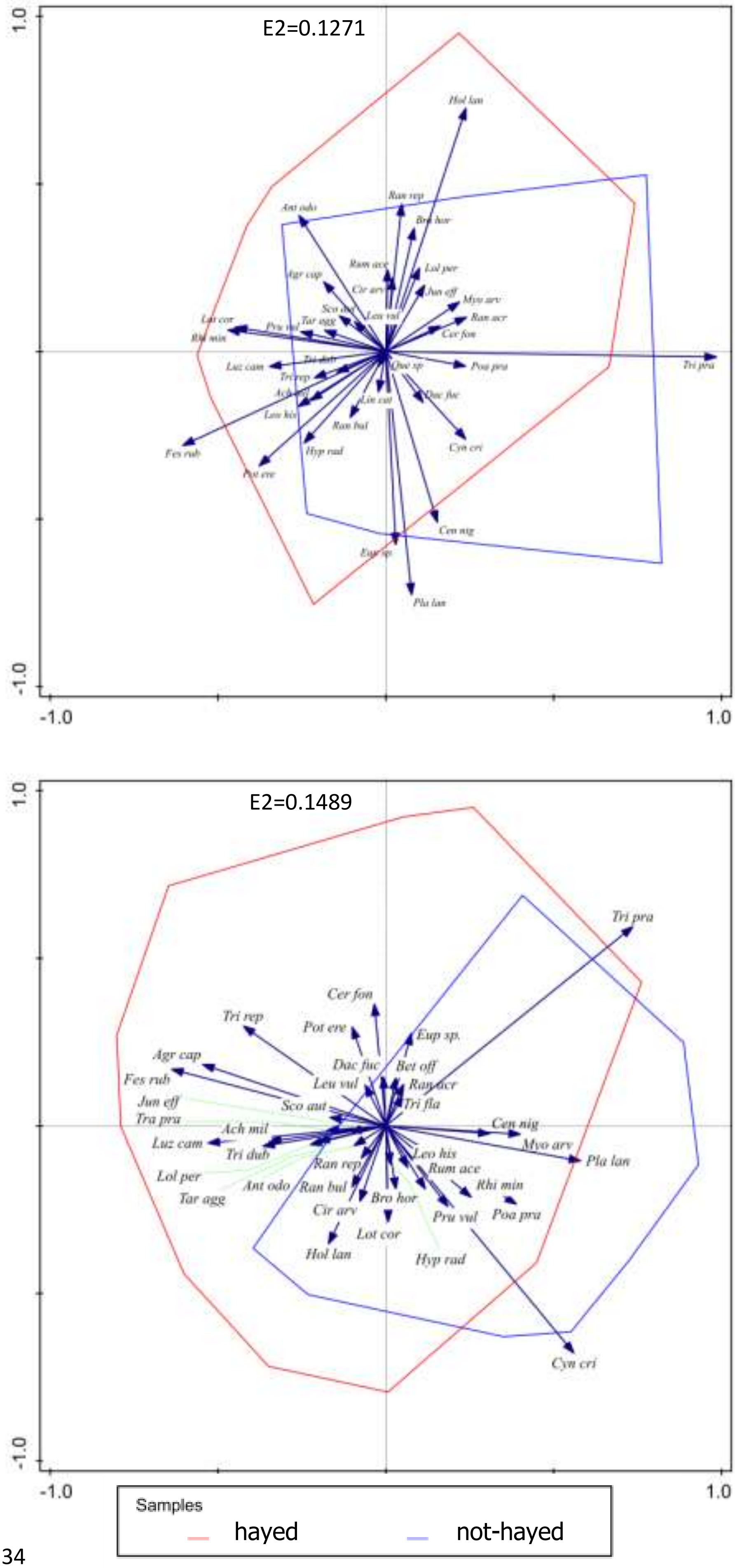

**(a)**
**2012**

E1=0.4528




**(b)**
**2013**

E1=0.5130




**(c) 2014**

E1=0.3673

**(d) 2015**

E1=0.2689

Figure 4.11: PCA biplots of the species and samples data for Cae Gross (a) CG2012, (b) CG2013, (c) CG2014 and (d) CG2015.

#### 4.3.5.3 ANOVA of PCA centroid scores of the survey data

A summary of the differences between groups coded for treatment and year together – i.e. Pi2011 (source), CG2011 (baseline receiver), CG2012 hayed, CG2012 not-hayed etc., as per the centroid ordination diagram (Figure 4.8), is presented in Table 4.9. *Post hoc* Tamhane tests following a one-way ANOVA [$F$(9,306) = 9.834, p <0.001] showed that the source and the baseline receiver (homogenous subset 'a') did not have significantly different ordination scores on axis 1. The hayed and not-hayed groups from 2015 (homogenous subset 'b') did not have significantly different ordination scores to each other, but did have significantly different scores to all other groups (Table 4.9). The not-hayed quadrats from 2012 and the hayed quadrats from 2013 had significantly different scores to each other and to all other groups and were the most distant on axis 1 to the source and baseline. The hayed quadrats from 2012, the not-hayed quadrats from 2013 and both groups from 2014 (homogenous subset 'c') were not significantly different to each other, but were to all other groups. There is no overlap between the subsets (no category is in more than one homogenous subset). In summary, hayed and not-hayed are significantly different in 2012 and 2013, but not in 2014 or 2015. Partial Eta squared suggests that 82.8% of the variation in the data was due to differences between the groups; this is supported by the large $F$ ratio (163.926).

Table 4.9: Results of comparison of PCA axis 1 centroid scores for CG and Pi2011

| Treatment | Mean | S.E. | df | MS | *F* | p | Partial Eta$^2$ |
|---|---|---|---|---|---|---|---|
| Pi2011 (source) | -0.886[a] | 0.015 | 9, 316 | 29.080 | 163.926 | <0.001*** | 0.828 |
| CG2011 (receiver baseline) | -0.757[a] | 0.043 | | | | | |
| CG2015 Not-hayed | -0.413[b] | 0.054 | | | | | |
| CG2015 Hayed | -0.411[b] | 0.061 | | | | | |
| CG2014 Hayed | -0.027[c] | 0.052 | | | | | |
| CG2014 Not-hayed | -0.012[c] | 0.074 | | | | | |
| CG2012 Hayed | 0.118[c] | 0.077 | | | | | |
| CG2013 Not-hayed | 0.259[c] | 0.143 | | | | | |
| CG2012 Not-hayed | 1.188[d] | 0.112 | | | | | |
| CG2013 Hayed | 2.091[e] | 0.129 | | | | | |

Notes:

*** indicates where significance levels are p <0.001. Treatment means with the same label (a-e) are not significantly different from one another (Tamhane; p<0.05).

### 4.3.6 Summary of the main results

- 22 species were recorded at the source meadow (Pikes Farm), but not at the baseline receiver (Cae Gross) (i.e. were not present) before treatment. Of these: 2 were undesirable and 5 were neutral; 7 were on the list of poor performing species from Chapter 1, Table 1.1.
- 10 of these missing species were transferred (by 2015) – all of them desirable species. 3 of these were on the list of poor performing species from Chapter 1, Table 1.1.
- 4 desirable species did not transfer (3 of which are MG5 species – constancy I and II) (and 7 other species, 2 of which are MG5 species – constancy I and IV).

- 12 species increased in the receiver after treatment – 9 desirable and 1 neutral species.
- 9 species decreased – 6 desirable species, 3 undesirable.
- The factorial repeated measures ANOVA showed that there was a statistically significant main effect of haying on the mean percentage cover of the desirable species, the hayed treatment having a higher mean percentage cover of desirable species than the not-hayed treatment.
- Except for 2013, the hayed group had a higher mean and total number of species than the not-hayed.
- 2015 had the highest mean and total number of species compared with all other years.
- The hayed quadrat group in 2015 was the most diverse and had the equal second highest evenness and equal lowest dominance – although the most dominant species was different to the source meadow.
- The source meadow most closely matched MG5a, the baseline receiver – MG6b and all post-treatment groups matched an MG5 type – all the hayed groups most closely matched MG5c (not a), although they had a closer match to MG5a than the source meadow.

## 4.4 Discussion

### 4.4.1 Introduction of additional species

There were 22 species that were present at the source meadow and not at the baseline receiver – i.e. they were missing from Cae Gross compared to a local semi-natural species-rich meadow. The percentage frequencies in the source meadow of the species that did transfer appear to be generally higher (walkover- 72%; mean: 18.89%) than those of the species that did not transfer (walkover- 26%; mean: 10.00%). Four species (*Carex flacca, Myosotis arvensis, Leontodon hispidus* and *Stellaria graminea*) established in the first year after strewing, *M. arvensis* only in not-hayed areas and *L. hispidus* in both hayed and not-hayed areas. However, both these species have small seeds (Grime *et al*., 1988), which may have either blown into the not-hayed area or stuck to the machinery and been transferred in this way.

*M. arvensis* was recorded in every post-treatment year and had a higher percentage frequency each year than that recorded at the source, suggesting that conditions (for example, lack of competition, amount of disturbance, soil moisture levels) were more favourable for this species at the receiver than at the source. *M. arvensis* is an autumn germinating annual, associated with disturbed habitats or bare ground with a R/SR strategy (Grime *et al*., 1988), suggesting a certain amount of disturbance or incomplete cover by the sward at the receiver (and not in the source).

*L. hispidus* was also recorded in every post-treatment year, but at a lower percentage frequency than that at the source. This may be due to the conditions at the receiver or to the time taken for the population to increase from establishment. As *L. hispidus* is a stress-tolerator associated with relatively undisturbed and infertile habitats and relatively high floristic diversity (Grime *et al*., 1988), unlike *M. arvensis,* this could suggest more disturbance at the receiver than at the source. Smith *et al.* (2000) found infrequent establishment of *L. hispidus* although other studies have not found difficulties: Trueman and Millett (2003) report success in five out of five attempts, recording it regularly across each introduction site. It often behaves as a colonizer (Grime *et al.,* 1988), therefore it would not be expected to be difficult, unless the sward is closed.

*C. flacca* and *S. graminea* were only recorded on the walkover. *S. graminea* was not recorded again, but *C. flacca* was recorded in 2013, at a higher percentage frequency than the source, although it was not recorded subsequently. *Carex flacca* has been found to be a difficult species to establish in new sites in a number of studies (e.g. Pakeman *et al.,* 2002; Holzel and Otte 2003; Donath *et al.,* 2007). There are a number of potential reasons for this, for example, their regeneration is mainly vegetative, with seedlings rarely seen in the field (Grime *et al.,* 1988). They also need long warm and moist periods (Schutz, 2000; Holzel and Otte, 2004), fluctuating temperatures (Schutz, 1998) stratification (Kettenring and Galatowitsch, 2007) and scarification (Schutz, 2000) and sometimes have low seed viability (Patzelt *et al.,* 2001). It may also be that, because they set seed early, the seed is already lost from the seed head when the hay is cut, making transfer using green hay more difficult (Holzel and Otte, 2003; Donath *et al.,* 2007). Trueman and Millett (2003) report some success with the species (one successful transfer in one attempt) although it was slow to establish and only recorded rarely and sporadically, as happened in this experiment.

Two more species (*Dactylorhiza fuchsii* and *Linum catharticum*) established in the second year after strewing (2013), *D. fuchsii* in not-hayed areas and *L. catharticum* in both hayed and not-hayed areas. *D. fuchsii* has dust-like seed, which may have been blown into the not-hayed areas. It may also have established due to previous green hay strewing in this field, since two years after strewing is very soon for this species to be recorded. Orchids are thought to take a number of years to establish after sowing (Trueman and Millett, 2003). *D. fuchsii* was recorded every subsequent post-treatment year, although at a lower percentage frequency than at the source. *L. catharticum* was recorded in the following year, but not 2015, at a higher percentage frequency than the source, having only been recorded on the walkover at Pikes Farm.

*L. catharticum* was found to perform poorly by Pywell *et al.* (2003) and also by Trueman and Millett (2003). It is a stress-tolerant, annual or biennial ruderal, which although associated with open swards in grasslands, needs perennial plants to provide some cover for successful germination and seedling survival (Grime *et al.*, 1988). The requirements of this species (along with the indications from *M. arvensis*) suggest an open sward at the receiver, more so than at the source. This could be due to the initial disturbance caused by the harrowing in preparation for the strewing and also by the annual cut and collect along with the aftermath grazing. Grazing is known to create gaps in the sward through the trampling action of the animals' feet (Bullock *et al.,* 2001). As management at Pikes Farm (the source meadow) is only through an irregular cut and collect, this might explain the difference between the frequencies of these species.

Two more species (*Lathyrus pratensis* and *Trisetum flavescens*) were recorded three years after strewing (2014). Both species were only recorded on the walkover (and so at a lower percentage frequency than at Pikes) and *L. pratensis* was not recorded in 2015. Trueman and Millett (2003) report success in three out of six attempts with *L. pratensis,* the species generally being recorded regularly, but less frequently than at the source, and success in five out of six attempts with *T. flavescens*, at slightly fewer or similar frequencies than at the source.

The final two introduced species were recorded in 2015. *Betonica officinalis* was recorded in one quadrat in a hayed area and *Platanthera chlorantha* (Figure 4.10), another orchid species, was recorded on the walkover. As an orchid species, this was recorded early, after hay strewing, compared to other studies (Trueman and Millett, 2003; Kotilinek *et al.,* 2015). *Betonica officinalis* is a stress-tolerator, typically associated with species-rich vegetation, although dispersal is thought to be poor (Grime *et al.,* 1988). It is slow-growing (Grime *et al.,* 1988), which may explain its late appearance. It is a late-flowering species (Grime *et al.,* 1988), which means it may be surprising that is has been successfully transferred in this experiment and may also explain the lack of success in other experiments (Trueman and Millett, 2003).

### 4.4.2 Introduction of other species

Three species were recorded after treatment, but not before treatment nor in the source meadow. Of these, *Tragopogon pratensis* could be said to be an opportunist species, being found in a range of grassland habitats (Grime *et al.,* 1988), and another, *Galium palustre*, has a preference for wet/damp conditions (Grime *et al.,* 1988). The appearance of this latter species may be a reflection of changing soil moisture levels in the meadow – particularly related to the very wet weather in 2012 (Meteorological Office, 2015).

### 4.4.3 Species that did not transfer

Of the 11 species that did not transfer, *Conopodium majus* had the highest percentage frequency (46%) in the source meadow. However, this species flowers relatively early (Grime *et al.,* 1988), which may have meant that the seed had been lost before the green hay was cut. Trueman and Millett (2003) report success with the species in four out of six attempts, although it was slow to establish and was only recorded at low frequencies. Losvik and Austad (2002) were also not successful in establishing *C. majus* when sowing a local seed/chaff mixture to a power harrowed receiver meadow, whereas Smith *et al.* (2000) found that it appeared after four years, but was infrequent in the vegetation. Kirkham *et al.* (2013) were not successful in transferring *C. majus* to their receiver site, when using green hay strewing, in spite of it being the most abundant species at the source site. They suggest that either the weather (*C. majus* having a chilling requirement for germination (Grime *et al.,* 1988)) or the ground conditions were not suitable for the establishment of this species, although they do note that other species with a chilling requirement were successful. Critchley *et al.* (2007) suggest a detrimental effect of spring-grazing on this early flowering species, although Hulme *et al.* (1999) found that *C. majus* increased in all grazing and exclusion of grazing treatments.

The remaining species that did not transfer all had percentage frequencies of ≤ 12% at the source (the species with a 12% frequency was *Juncus articulatus,* which is undesirable). The mean percentage frequency in the source meadow was 10% compared to 19% for the species that did transfer. It is surprising that *Dactylis glomerata* did not transfer, as, although it was only found in the source meadow at low frequency, it is a generalist and competitive species (Grime *et al.,* 1988). However, it is also one of the species that appeared in the list of poor performing species in Chapter 1 (Table 1.1).

Of the remaining desirable species, *Carex* species are known to be difficult (4.4.1), possibly because they spread more by vegetative means than by seed (Grime *et al.,* 1988) and this plant was only recorded at 2% frequency (i.e. in one quadrat) in the source; *Crepis capillaris* was only present at 4% frequency; *Heracleum sphondylium* and *Silene flos-cuculi* were only seen on the walkover of the source meadow and *S. flos-cuculi* has a preference for damp conditions, which were probably not present in the receiver meadow, at the locations where the seed fell (although this is in contrast to *Galium palustre*, above).

In summary, of the four desirable species that did not transfer, two prefer wet/damp conditions (and are not MG5 species), one flowers early and two were only present at low frequencies in the source. A third species only present at low frequencies (≤2%) was one of those that prefer damp/wet conditions. The remaining desirable species was *Crepis capillaris*, which was only present at the source at a relatively low frequency.

### 4.4.4 Changes in frequencies of existing species

The species that increased substantially had the second highest percentage frequency (59.67%; Table 4.4d) in the source meadow of the five groups of species, the highest being the group of species that did not change in frequency substantially (61.67%; Table 4.4f). These 12 species (Table 4.4d) are all desirable and are all MG5 constancy class III (i.e. ≥41-60% frequency), apart from *Euphrasia* sp., which is not an MG5 species (Rodwell, 1992). These species had frequencies of ≥12% in the source meadow and are relatively generalist species (excepting *Euphrasia* sp. and *Rhinanthus minor*), even though they are associated with habitats of high species-richness (Grime *et al.,* 1988).

The group of nine species that were present in the baseline receiver meadow, but decreased substantially after treatment, had the second highest mean percentage frequency in the source meadow (the highest being the group that were present in the baseline receiver meadow and increased after treatment). These nine species included three that were not present in the source meadow. Changing conditions in the receiver meadow may have led to a reduction in these species (e.g. soil moisture, increase in competition) and the six that were present in the source may also have failed to transfer, meaning no increase in numbers of plants of these species from seeds/seedlings from the source meadow. The species that decreased included *Lotus corniculatus,* which has been found to be difficult to establish in other studies: e.g. Smith *et al.* (2000; recorded only after six years); Hopkins *et al.* (1999; established but at low frequency); Besenyei (2000, little establishment), Hofmann and Isslestein (2004; low establishment rate), Rayner (2005; inconsistent establishment). Pakeman *et al*. (2002) had good establishment but suggest sowing this species as a later introduction. It can be low growing, with just a moderate number of large seeds produced infrequently (Grime *et al.,* 1988). The large seed size may mean that the seeds drop out of the hay before being transferred.

### 4.4.5 Differences between years

Once a species had been introduced, four out of 10 (*D. fuchsii, L. hispidus, M. arvensis, and T. flavescens*) were found in all subsequent years. *B. officinalis* and *P. chlorantha* were only found in the last year of quadrat surveys. *C. flacca* was recorded in 2012 and 2013 but not subsequently; similarly, *L. catharticum* was recorded in 2013 and 2014 and *L. pratensis* (2014) and *S. graminea* (2012) were only recorded in one year, on the walkover. This is similar to Smith *et al.* (2000) who found that changes in species-richness was intermittent, rather than an even increase over time and that changes were not always positive (e.g. desirable species increased and then decreased).

The mean percentage cover of desirable species increased over time, although the effect of year was not significant (but this was close, p = 0.036) according to the factorial repeated measures ANOVA. PCA analysis illustrates the differences between the years, although most of the polygons for each year overlap with most of the other years. Only CG2011 and CG2015 overlap with Pi2011. The one-way ANOVA of the PCA ordination scores found that differences between the some of the years were significant (e.g. CG2015 hayed is different CG2012-2014 hayed). The species on the overall biplot (Figure 4.9) with the longest arrows (best fit to the variation expressed by the ordination plot) are *R. minor* and *T. pratense,* suggesting that differences between the years may be primarily associated in some way with these key species. From the individual year ordination plots, *Trifolium pratense* appears to be more associated with the not-hayed quadrats, whereas *Rhinanthus minor* changes from being associated with the not-hayed quadrats in 2012, to the hayed quadrats in 2013 and 2014. Smith *et al.* (2000) also found that treatment effects changed over time. The most dominant species after treatment is never the same as in the source or CG2011. It is *R. minor* in 4/12 post-treatment groups and *T. pratense* in 5/12 post-treatment groups.

### 4.4.6 Differences between hayed and not-hayed treatment areas

Each year, the hayed quadrats were more similar to CG2011 and Pi2011 than the not-hayed quadrats and they are also more similar to an MG5 community than are the not-hayed quadrats of the same year (Section 4.3.4). Species diversity is higher in hayed than not-hayed in three out of four years (and total and mean number of species both tend to be higher in hayed) and the mean percentage cover of desirable species was significantly higher in hayed than in the not-hayed treatment, according to the repeated measures ANOVA.

Three of the introduced species (*Betonica officinalis, Carex flacca* and *Stellaria graminea*) appeared in the hayed areas first, two (*Dactylorhiza fuchsii* and *Myosotis arvensis,* both of which have light seed, discussed previously; Section 4.4.1) appeared in the not-hayed areas first, three (*Leontodon hispidus, Linum catharticum* and *Trisetum flavescens*) appeared in both areas and the remaining two were found on walkovers and were not attributed to a treatment.

In 2014 and 2015, the hayed and not-hayed quadrats became more similar to each other, as illustrated by the non-significant difference in target species between years. This was probably due to the traditional management activities of cutting, rowing and tedding leading to hay being spread from the treatment areas into the untreated area and *vice versa*. Contamination/ invasion of non-hayed areas by species from hayed areas is a common occurrence in field-based species introductions (Leps *et al.,* 2007;

Burmeier *et al.,* 2011; Auestad *et al.,* 2015) and has also been used to reduce the amount of seed/hay needed in introduction projects by strewing hay in narrow strips, so that the species will then disperse into the wider site (Burmeier *et al.,* 2011; Tierney pers. comm., 2011), although spread of these species can be slow (Hedberg and Kotowski, 2010).

## 4.5 Conclusions

The species that were missing from Cae Gross compared to Pikes Farm SSSI and therefore the target species for the experiment, were: *Betonica officinalis, Carex flacca, Dactylorhiza fuchsii, Lathyrus pratensis, Leontodon hispidus, Linum catharticum, Myosotis arvensis, Platanthera chlorantha, Stellaria graminea, Trisetum flavescens, Carex* sp., *Conopodium majus, Crepis capillaris, Dactylis glomerata, Equisteum arvense, Heracleum sphondylium, Hypericum perforatum, Juncus articulatus, Juncus conglomeratus, Silene flos-cuculi* and *Rumex obtusifolius.* The first 10 of these species (nine desirable, one neutral) were transferred by this experiment and the latter 11 (four desirable, four neutral, two undesirable and one only identified to family level) were not. Seven of these species are on the list of poor performing MG5 species taken from a review of the literature (Chapter 1, Table 1.1). Orchids, of which there are two in the above list, are also identified in the literature as difficult species to establish in created meadows.

This experiment found that green hay strewing increased the number of species in an existing species-rich meadow and can also increase the frequency and abundance of existing species. It can also increase the similarity to Rodwell's definition of MG5 communities (Rodwell, 1992). Haying had a statistically significant effect on the mean percentage cover of desirable species, the hayed treatment having a higher mean percentage cover of desirable species than the not-hayed treatment (in all years).

Overall, species that did transfer had a higher percentage frequency in the source meadow than those that did not, although some individual species of low frequency did transfer and some of high frequency did not. This suggests that strewing green hay onto an existing species-rich meadow is a viable technique to introduce missing species, although some individual species may need other techniques, such as hand collection and sowing of seed, or introduction of plug plants for successful establishment. It also seems that traditional management techniques enhanced the benefit of adding green hay in smaller areas, as the seeds were distributed more evenly over time.

Figure 4.10: a-c: *Platanthera chlorantha* and the post-treatment vegetation in the receiver meadow Cae Gross (25.6.15).

# Chapter 5

# Enhancing an existing created meadow using green hay strewing and disturbance

## 5.1 Introduction

Successful establishment of new plants, whether of new species or new individuals of existing species, depends on the availability of suitable microsites within the grassland (Foster, 2001; Hofmann and Isselstein, 2004; Walker *et al.,* 2004; Edwards *et al.,* 2007). In addition to the introduction of missing species into species-rich grassland, the creation of gaps in the sward is known to be important to allow the recruitment of seedlings to the community (Bischoff, 2000; Smith *et al.,* 2000; Turnbull *et al.,* 2000; Bullock *et al.,* 2001). Competition from existing vegetation and the presence of a litter layer can inhibit seedling establishment (Foster and Gross, 1998; Donath *et al.,* 2006; Ruprecht *et al.,* 2010). However, the harsh conditions present in bare soil can also be unfavourable for successful germination and survival (Hutchings and Booth, 1996). Disturbed areas within existing vegetation provide intermediate conditions between these two extremes, but these need to be created using methods that are applicable on a large scale (Rayner, 2005). These gaps can be created in a number of ways: for example, through trampling by grazing animals (Bullock *et al.,* 2001) and via artificial means, such as unauthorized motorcycling (Trueman and Millett, 2003) or agricultural machinery, such as power harrows (Hofmann and Isselstein, 2004; Edwards *et al.,* 2007).

Grazing is part of the traditional management of hay meadows – the removal of aftermath grazing results in changes in species composition and a decline in species richness (Kirkham *et al.,* 1996). Grazing acts on species composition in several ways, including: the action of trampling creating gaps in the vegetation (allowing colonization); the effect of selective grazing (leading to the decline of preferentially grazed species) and the maintenance of the vegetation, meaning that it does not become rank, which would favour more competitive species (Bullock *et al.,* 2001). Timing and intensity of grazing are both important (Crofts and Jefferson, 1999; Bullock *et al.,* 2001; Rodwell *et al.,* 2007).

The timing of grazing varies according to various factors including location, farming system and hydrology (Crofts and Jefferson, 1999). In some areas, such as upland valleys, meadows are used for spring grazing by ewes and lambs, after which the meadows are shut up for hay, hay is cut in mid-July and the meadow is grazed again in late summer and autumn (*Ibid.*; Rodwell *et al.,* 2007). Some studies have found spring-grazing to be beneficial to species diversity (Gibson *et al.,* 1987; Smith *et al.,* 2000), whilst others have found it to be detrimental (Critchley *et al.,* 2004; DEFRA, 2004; Jefferson, 2005). These differences may be related to grazing intensity and the date when animals are removed from the meadow (Rinella and Hileman, 2009), for example, Critchley *et al.* (2007) found detrimental effects of spring-grazing when it was prolonged, although the effect of grazing depends on the species, both of plant and grazing animal (Crofts and Jefferson, 1999;

Bullock *et al.,* 2001; Rinella and Hileman, 2009). For example, *Rhinanthus minor* is susceptible to heavy grazing during spring, although grazing can be used to check the growth of *Senecio* spp. (Crofts and Grayson, 1999). Lowland meadows are also grazed after hay cutting, but spring grazing is unusual as it has a detrimental effect on the hay cut and also on early flowering species (Crofts and Jefferson, 1999). Further work is needed to understand the optimal intensity and timing of grazing within species-rich meadows (Rodwell *et al.,* 2007).

Aim:

To investigate the effect of disturbance (scarification with a power harrow) in combination with grazing and the use of strewn green hay from a species-rich source meadow, on to a species-enriched meadow with existing diversity; i.e. enhancement of an existing created meadow.

Objectives:

To apply three replicated disturbance treatments: no disturbance (no-disturbance), a small amount of disturbance (low-disturbance) and twice the amount of disturbance (high-disturbance) in combination with the haying treatment.

To compare the effect of grazing in the first autumn and spring with areas that were not grazed during this time.

To compare the vegetation in the receiver meadow before treatment (baseline) and in the different treatment areas, with that of the source meadow.

## 5.2 Methods

### 5.2.1 Site Descriptions

#### 5.2.1.1 Receiver meadow

Golden Field (SO339294) is a 1.84 ha hay meadow, on The Bryn farm, Upper Bryn, approximately 19 km south-west of Hereford (Figures 5.1, 5.2) and five km from the source meadow. The field slopes downhill towards the north and had a pre-existing green hay strewn strip (from 2010, added by the site owner, unconnected with this experiment) at the southern end, approximately 3 m wide, running east-west across the field (Tierney pers.comm., 2011; Figure 5.3). Its management is a cut and remove in July with aftermath grazing by sheep (Table 5.1). It is also grazed by ewes with lambs in spring, after which livestock are excluded, except in 2015, when the field was not spring-grazed.

In 2011, Golden Field had a total of 28 species and a mean number of species per quadrat of 14.12. Its Simpson's Index of diversity was 1.92 and, using MAVIS, it most closely matched the MG6b *Lolium perenne-Cynosurus cristatus, Anthoxanthum odoratum* sub-community. Species recorded at high frequencies included: *Festuca rubra, Rhinanthus minor, Trifolium repens, Anthoxanthum odoratum, Cynosurus cristatus, Holcus lanatus, Lolium perenne, Trifolium dubium* and *Ranunculus repens.*


Figure 5.1: Location of Golden Field (receiver meadow) and Three Yew Trees (source meadow). Locations are indicated with red markers, the most northerly being Three Yew Trees.


Figure 5.2: Location of Golden Field (outlined in green), The Bryn, Upper Bryn (outlined in red), Herefordshire (Digimap, 2015).

Figure 5.3: The pre-experiment vegetation in Golden Field (29.6.2011; facing north).

Table 5.1: Site history and management of Golden Field

| Year | Site History and Management |
|---|---|
| **Pre-2010** | Arable field, then a meadow |
| **2010** | One 3 m wide strip of green hay added at top (south end) of field (by site owner, unconnected to this experiment) |
| **2011** | Experiment laid down – i.e. addition of treatment strips including hayed strips (July) |
| | Top half autumn-grazed by sheep (September-November), lower half not grazed |
| **2012** | Top half spring-grazed by sheep (May) |
| | Hay cut in early August |
| | Whole field autumn-grazed by sheep (September-November) |
| **2013** | Whole field spring-grazed by sheep (May), field limed |
| | Hay cut in early August |
| | Whole field autumn-grazed by sheep (September-November) |
| **2014** | Whole field spring-grazed by sheep (May) |
| | Hay cut in early August |
| | Whole field autumn-grazed by sheep (September-November) |
| **2015** | No spring-grazing |
| | Hay cut in early August |
| | Whole field autumn-grazed by sheep (September-November) |

### 5.2.1.2 Source meadow

Three Yew Trees (SO335341), located 18 km south-west of Hereford (Figure 5.4), has several species-rich meadows, of a neutral dry grassland type, which are managed as traditional hay meadows (i.e. a cut and remove in July with aftermath grazing by cattle). Of these, three small neighbouring meadows, totalling 1.2 ha, were used as the source.

In 2011, Three Yew Trees has a total of 41 species and a mean number of species per quadrat of 16.00. Its Simpson's Index of diversity was 1.80 and, using MAVIS, it most closely matched the MG5 *Cynosurus cristatus-Centaurea nigra* community type. Species present included: *Rhinanthus minor, Centaurea nigra, Cynosurus cristatus, Primula veris*, *Leontodon hispidus* and two orchid species: *Dactylorhiza fuchsii* and *Neottia ovata* (Figure 5.5).

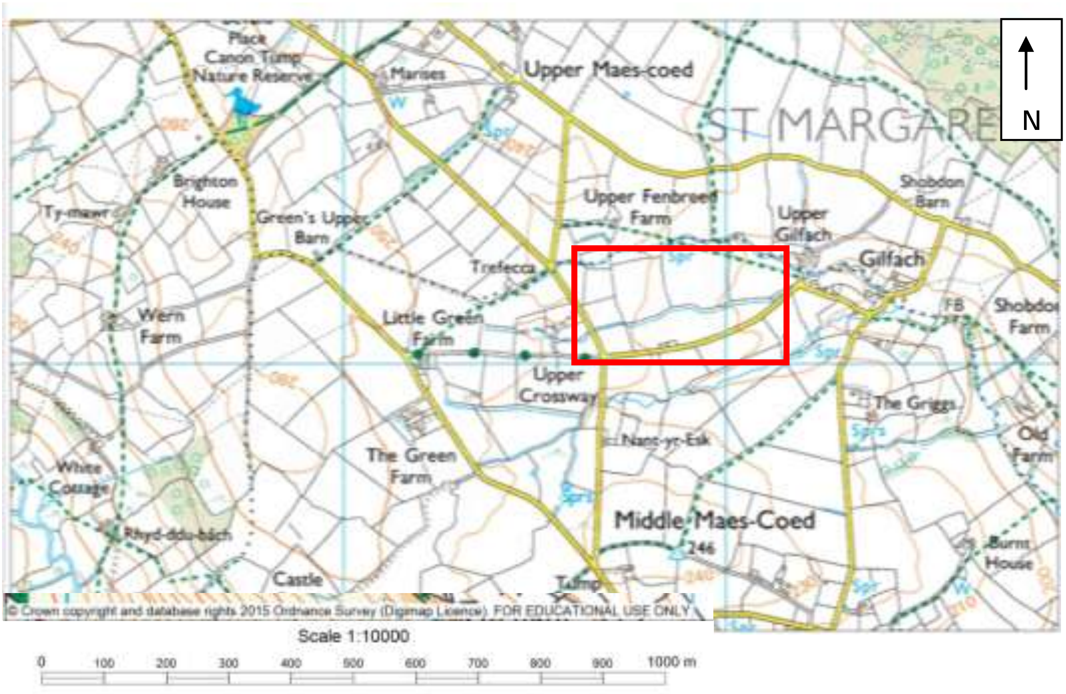



Figure 5.4: Location of Three Yew Trees (Digimap, 2015).

Figure 5.5: The vegetation in the source meadow at Three Yew Trees (30.6.2011), facing east.

### 5.2.1.3 Comparison of source and receiver sites

Twenty-three species were present in 3YT but not in GF2011, 19 of which were desirable. For more details, see the results section and Table 5.4. Of these 23, three were orchids (although not MG5 species), one (*Dactylis glomerata*) is an NVC constancy class IV (61-80% frequency) species, four are constancy III (41-60%), seven are constancy II (21-40%), four are constancy I (0-20%) and the remaining four are not MG5 species (*Stellaria graminea, Ajuga reptans, Hypericum perforatum* and *Polygala vulgaris*, all considered desirable species in this study).

The species that are present in the source meadow, but absent from the baseline receiver, and therefore the target species for this experiment, are:

*Achillea millefolium, Ajuga reptans, Anacamptis morio, Briza media, Carex flacca, Conopodium majus, Dactylis glomerata, Dactylorhiza fuchsii, Heracleum sphondylium, Hypericum perforatum, Lathyrus pratensis, Leontodon hispidus, Leucanthemum vulgare, Neottia ovata, Poa pratensis, Polygala vulgaris, Potentilla erecta, Primula veris, Prunella vulgaris, Ranunculus bulbosus, Stellaria graminea, Trisetum flavescens* and *Vicia cracca.* Of these, *Briza media, Carex flacca, Conopodium majus, Dactylis glomerata, Heracleum sphondylium, Lathyrus pratensis* and *Vicia cracca* are on the list of poor performing MG5 species from a review of the literature (Chapter 1, Table 1.1). Orchids are also identified in the literature as difficult species to establish in created meadows.

### 5.2.2 Experimental design

In July 2011, the receiver meadow was cut and treatments of no-disturbance (control), low-disturbance and high-disturbance (using a power harrow) in combination with hayed and not-hayed treatments, were applied in 3 m wide strips (Figure 5.5). Low-disturbance was created by applying three passes of the harrow and high-disturbance was six passes of the harrow (Section 2.3, Figure 2.1), the day before the hay was strewn. The machinery was set to create disturbance in the thatch, but minimal disturbance of the soil, i.e. to a maximum depth of about 1 cm. The vegetation cover was approximately 25% after the low disturbance treatment and 50% after the high disturbance treatment. The location of the treatments within the design was determined using random numbers and three replications were created. On 28th July, the source meadow was cut (at a height of 2-3 cm) and baled and the bales were transported to the receiver meadow, rolled out by hand and spread using a tedder, on the same day. The green hay application rate was 1:3.

The southern end of the field was grazed in autumn 2011 and spring 2012, but the northern end was not grazed (Figure 5.5). In 2013 and 2014, the whole field was grazed in autumn and then in spring. In 2015, the whole field was not spring-grazed, but was autumn-grazed. This was not part of the original layout of the experiment, but was added by the site owner as he needed to graze some animals here. The orientation was chosen due to the location of gates for access to the field by the animals and also meant that, if necessary, the grazed end of the field could be excluded from the experiment, with no loss of plots.

The experimental site was surveyed in the summers of 2012-2015 (Tables 5.2 and 5.3), with six quadrats surveyed in each strip, three in the grazed portion of the field and three in the not-grazed, using random numbers to locate quadrats along a transect in the middle of the strip. Fifty quadrats were surveyed in both the source and the receiver in July 2011 (pre-treatment), randomly located across the sites (Tables 5.2 and 5.3). In the first four years after treatment, 108 quadrats were surveyed at the receiver site, with six quadrats surveyed in each strip, three in the grazed portion of the field and three in the not-grazed, using random numbers to locate quadrats along a transect in the middle of the strip. These were carried out in June 2012-2015. During these surveys any additional species, outside the quadrats, were also recorded as a species list. A walkover survey was carried out on 29th June 2016, walking across as much of the site as possible, in a W pattern, listing all species seen.

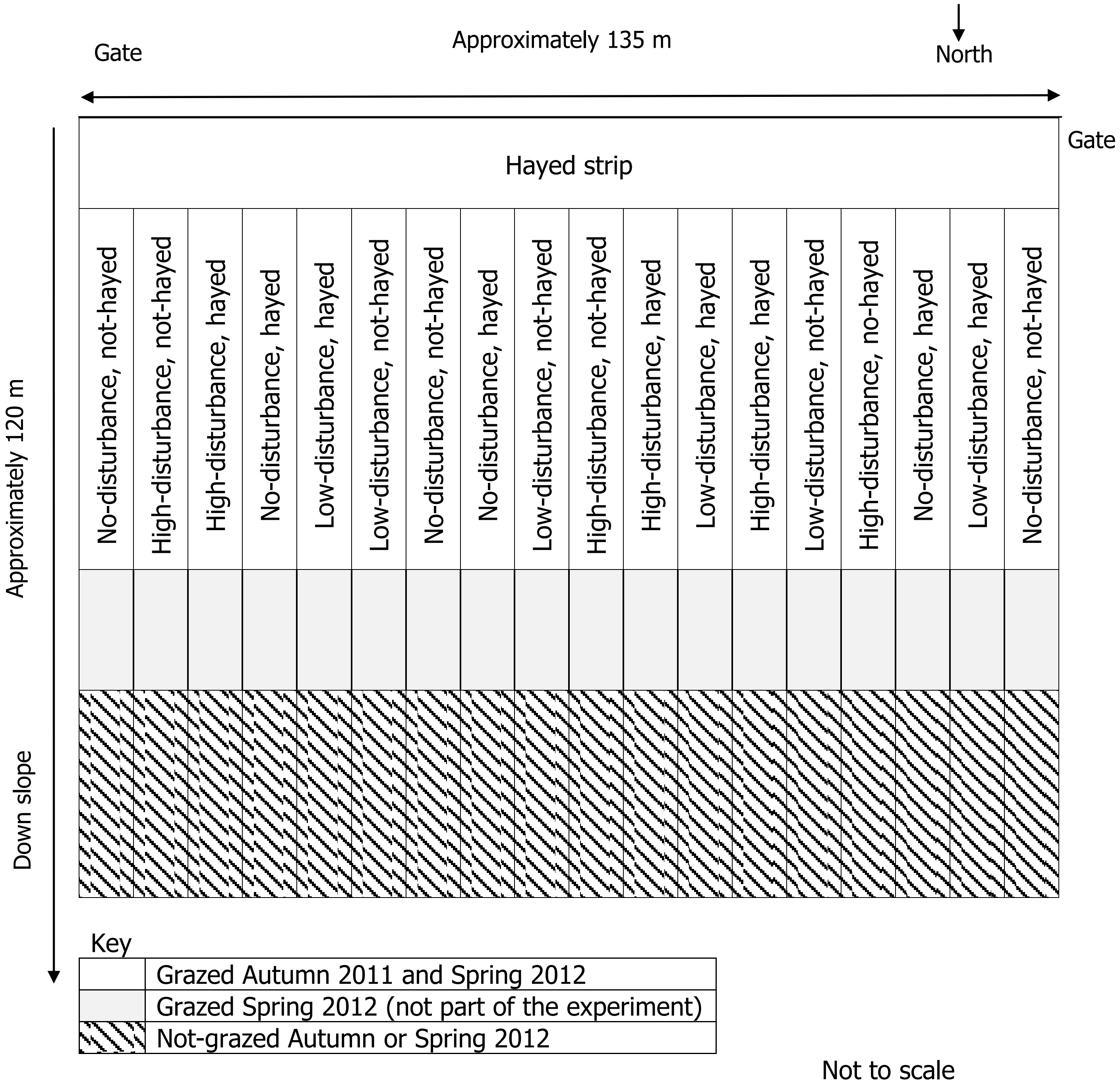



Figure 5.5: Experimental layout at Golden Field.

Table 5.2: Survey dates for Golden Field (GF) and its source meadow

| **Date** | **Meadow** |
|---|---|
| 30.6.11 | Source meadow (Three Yew Trees) |
| 29.6.11 | GF baseline |
| 20.6.12<br>23.6.13<br>22.6.14<br>21.6.15<br>29.6.16 | GF post-treatment |

Table 5.3: Datasets from vegetation surveys

| **Dataset** | **Number of quadrats** | |
|---|---|---|
| | **2011** | **2012-15** |
| Three Yew Trees, 2011 | 50 | N/A |
| Golden Field, The Bryn | | |
| All quadrats | 54 | 108 |
| Golden Field not-hayed pre-2012 | 45 | N/A |
| Golden Field original haying (pre-2012) | 9 | |
| All quadrats, grazed | N/A | 54 |
| No-disturbance, not-hayed, grazed | | 9 |
| Low-disturbance, not-hayed, grazed | | 9 |
| High-disturbance, not-hayed, grazed | | 9 |
| No-disturbance, hayed, grazed | | 9 |
| Low-disturbance, hayed, grazed | | 9 |
| High-disturbance, hayed, grazed | | 9 |
| All quadrats, not-grazed | | 54 |
| No-disturbance, not-hayed, not-grazed | | 9 |
| Low-disturbance, not-hayed, not-grazed | | 9 |
| High-disturbance, not-hayed, not-grazed | | 9 |
| No-disturbance, hayed, not-grazed | | 9 |
| Low-disturbance, hayed, not-grazed | | 9 |
| High-disturbance, hayed, not-grazed | | 9 |

### 5.2.3 Data preparation and analysis

#### 5.2.3.1 Significance testing of treatment effects

A factorial repeated measures ANOVA was conducted to determine the effects of year (4), haying (2), grazing (2) and disturbance (3) levels on the mean percentage cover of the desirable species (following Laerd Statistics, 2013). The source and the baseline were not included in this analysis.

#### 5.2.3.2 Other analyses

Similar analyses to those done on the Castle Vale and Cae Gross data were carried out on the data for this experiment, e.g. mean and total number of species comparisons, comparisons of species diversity measures and Canoco (PCA). However, they are not included here, due to word count constraints, and the focus of the study being on the transfer of species from the source meadow rather than on the community as a whole. The other analyses are within Appendices 5.2-5.6.

## 5.3 Results

### 5.3.1 Comparison of species in the source and receiver meadows before and after treatment

#### 5.3.1.1 Golden Field baseline (GF2011) and after treatment (GF2012-2015) – comparing hayed and not-hayed

Eight species that were not found in the receiver meadow before treatment, but did occur in the source meadow, were found in the receiver meadow after treatment, seven of which are desirable (Tables 5.4, 5.5). These were three constancy class III species, three constancy II species and two that are not MG5 species but are both found in meadows: *Dactylorhiza fuchsii* and *Stellaria graminea* (the one neutral desirability species of the eight species that transferred). Their percentage frequencies in the source meadow ranged from 100% (*Leontodon hispidus*) to 4% (*Stellaria graminea*; Table 5.4). *Stellaria graminea* was only found in a hayed area (Table 5.5, Appendix 5.1). *Trisetum flavescens* was first found in 2013, only in the hayed treatment, and was then found in 2014 at higher percentage frequency in the not-hayed area (6% (not-hayed) versus 2% (hayed)). The mean percentage frequency in the source of the eight species that did transfer was 32.5%. *Heracleum sphondylium* is one of the poor performing species and *Dactylorhiza fuchsii* can also be considered a difficult species.

Fifteen species were found in the source meadow but not in the receiver before or after treatment, 12 of which are desirable (Table 5.4). Their frequencies in the source meadow ranged from 58% (*Conopodium majus*) to

Table 5.4: A comparison of the species and their percentage frequencies, recorded in the source and Golden Field in each year

w/o indicates species seen on a walkover of the field but not recorded in a quadrat. Text in red highlights differences in presence/absence between meadows or years

| | Percentage frequencies | | | | | | MG5 Constancy and Desirability | |
|---|---|---|---|---|---|---|---|---|
| | 3YT2011 | 2011 | 2012 | 2013 | 2014 | 2015 | | |
| **5.4a Species present in source and post-treatment receiver but absent from the baseline receiver (i.e. species that were transferred by the treatment in this experiment)** | | | | | | | | |
| *Dactylorhiza fuchsii* | 56 | **0** | 0 | 0 | 0 | **1** | - | D |
| *Heracleum sphondylium* | 18 | **0** | **6** | 3 | 3 | 1 | II | D |
| *Leontodon hispidus* | 100 | **0** | **2** | 5 | 7 | 3 | II | D |
| *Poa pratensis* | 34 | **0** | **50** | 95 | 74 | 91 | II | D |
| *Prunella vulgaris* | 10 | **0** | **18** | 31 | 49 | 66 | III | D |
| *Ranunculus bulbosus* | 8 | **0** | 0 | 0 | **1** | 10 | III | D |
| *Stellaria graminea* | 4 | **0** | 0 | 0 | 0 | **1** | - | N |
| *Trisetum flavescens* | 30 | **0** | 0 | **3** | 4 | **0** | III | D |
| Mean percentage frequency in the source | 32.5 | | | | | | | |
| **5.4b Species that were never found in the receiver meadow, but were in the source** | | | | | | | | |
| *Achillea millefolium* | 32 | **0** | 0 | **0** | 0 | 0 | III | D |
| *Ajuga reptans* | 2 | **0** | 0 | 0 | 0 | 0 | - | D |
| *Briza media* | 20 | **0** | 0 | 0 | 0 | 0 | II | D |
| *Carex flacca* | 2 | **0** | 0 | 0 | 0 | 0 | I | D |
| *Conopodium majus* | 58 | **0** | 0 | 0 | 0 | 0 | I | D |
| *Dactylis glomerata* | 40 | **0** | 0 | 0 | 0 | 0 | IV | N |
| *Hypericum perforatum* | w/o | **0** | 0 | 0 | 0 | 0 | - | N |
| *Lathyrus pratensis* | 8 | **0** | 0 | 0 | 0 | 0 | II | D |
| *Leucanthemum vulgare* | 2 | **0** | **0** | 0 | 0 | 0 | II | D |
| *Neottia ovata* | w/o | **0** | 0 | 0 | 0 | 0 | - | D |
| *Anacamptis morio* | 38 | **0** | 0 | 0 | 0 | 0 | - | D |
| *Polygala vulgaris* | w/o | **0** | 0 | 0 | 0 | 0 | - | D |
| *Potentilla erecta* | 4 | **0** | 0 | 0 | 0 | 0 | I | D |
| *Primula veris* | w/o | **0** | 0 | 0 | 0 | 0 | II | D |
| *Vicia cracca* | 42 | **0** | 0 | 0 | 0 | 0 | I | N |
| Mean percentage frequency in the source (excluding species only recorded on walkover) | 22.55 | | | | | | | |
| **5.4c Species present in post-treatment receiver that were not recorded before treatment nor in the source** | | | | | | | | |
| *Agrostis stolonifera* | 0 | 0 | **3** | 2 | **0** | **3** | I | U |
| *Alopecurus geniculatus* | 0 | 0 | 0 | 0 | **1** | 4 | - | U |
| *Arrhenatherum elatius* | 0 | 0 | **2** | 0 | 0 | 0 | II | N |

(cont.)

| | Percentage frequencies | | | | | | MG5 Constancy and Desirability | |
|---|---|---|---|---|---|---|---|---|
| | 3YT2011 | 2011 | 2012 | 2013 | 2014 | 2015 | | |
| *Betula* sp. seedling | 0 | 0 | 0 | **1** | 1 | **0** | - | U |
| *Cardamine pratensis* | 0 | 0 | **5** | 6 | 6 | 4 | I | D |
| *Corylus avellana* seedling | 0 | 0 | 0 | 0 | 0 | **1** | - | U |
| *Holcus mollis* | 0 | 0 | **1** | 0 | 0 | 0 | - | U |
| *Juncus effusus* | 0 | 0 | 0 | 0 | **1** | **0** | I | N |
| *Juncus bufonius* | 0 | 0 | 0 | **3** | 2 | **0** | - | N |
| *Prunus spinosa* seedling | 0 | 0 | 0 | 0 | 0 | **1** | - | U |
| *Rumex crispus* | 0 | 0 | 0 | **10** | 8 | 8 | - | U |
| *Vicia sativa* | 0 | 0 | **1** | 1 | 2 | **0** | - | N |
| *Vicia sepium* | 0 | 0 | 0 | 0 | 0 | **6** | - | N |
| (Bare ground) | 0 | 0 | 0 | **1** | 3 | 1 | - | - |
| **5.4d Species that increased substantially after treatment** | | | | | | | | |
| *Agrostis capillaris* | 96 | 9 | 49 | 77 | 44 | 48 | IV | D |
| *Bromus hordeaceus* | 6 | 43 | 43 | 97 | 97 | 94 | I | N |
| *Centaurea nigra* | 70 | 2 | 6 | 10 | 17 | 22 | IV | D |
| *Cerastium fontanum* | 30 | 72 | 71 | 87 | 65 | 99 | II | D |
| *Hypochaeris radicata* | 38 | 11 | 13 | 19 | 28 | 37 | III | D |
| *Scorzoneroides autumnalis* | 2 | 4 | 3 | 12 | 15 | 13 | III | D |
| *Plantago lanceolata* | 96 | 17 | 34 | 53 | 68 | 69 | V | D |
| *Ranunculus acris* | 84 | 41 | 88 | 94 | 97 | 99 | III | D |
| *Trifolium pratense* | 96 | 41 | 60 | 57 | 65 | 78 | IV | D |
| *Bellis perennis* | **0** | 22 | 44 | 68 | 76 | 65 | I | D |
| *Myosotis arvensis* | **0** | 4 | 20 | 75 | 30 | 47 | - | D |
| *Rumex obtusifolius*[a] | **0** | 4 | 19 | 3 | 4 | 2 | - | U |
| Mean percentage frequency in the source (excluding species not present in source) | 57.56 | | | | | | | |
| **5.4e Species that decreased substantially after treatment** | | | | | | | | |
| *Euphrasia* sp. | 28 | 70 | 1 | 2 | 5 | 4 | - | D |
| *Festuca rubra* agg. | 88 | 98 | 95 | 66 | 56 | 44 | V | D |
| *Lotus corniculatus*[b] | 84 | 2 | **0** | 0 | **3** | 2 | V | D |
| *Luzula campestris*[b] | 18 | 2 | **0** | 0 | 0 | **3** | III | D |
| *Phleum pratense*[b] | **0** | 56 | 7 | 42 | 31 | 41 | I | D |
| *Quercus* sp. | 2 | 2 | **0** | 0 | 0 | 0 | - | U |
| *Rhinanthus minor* | 98 | 98 | 43 | 60 | 27 | 54 | II | D |
| *Trifolium repens* | 36 | 98 | 19 | 31 | 83 | 79 | IV | D |
| Mean percentage frequency in the source (excluding species not present in source) | 50.57 | | | | | | | |

(cont.)

| | Percentage frequencies | | | | | | MG5 Constancy and Desirability | |
|---|---|---|---|---|---|---|---|---|
| | 3YT2011 | 2011 | 2012 | 2013 | 2014 | 2015 | | |
| **5.4f Species that did not change in frequency substantially** | | | | | | | | |
| *Anthoxanthum odoratum* | 100 | 96 | 100 | 99 | 96 | 100 | IV | D |
| *Cynosurus cristatus* | 8 | 100 | 98 | 100 | 100 | 100 | V | D |
| *Holcus lanatus* | 46 | 100 | 100 | 99 | 100 | 100 | IV | D |
| *Lolium perenne* | 2 | 100 | 95 | 94 | 86 | 93 | III | D |
| *Rumex acetosa* | 20 | 76 | 93 | 89 | 77 | 81 | III | D |
| *Taraxacum* spp. | 38 | 52 | 69 | 62 | 63 | 49 | III | D |
| *Trifolium dubium* | **0** | 100 | 53 | 99 | 95 | 100 | II | D |
| *Ranunculus repens* | **0** | 94 | 93 | 84 | 77 | 85 | I | U |
| Mean percentage frequency in the source (excluding species not present in source) | 35.67 | | | | | | | |

Notes:

[a] Species increased, then decreased back to initial levels
[b] Species decreased, then increased back to initial levels

2% (*Ajuga reptans*, *Carex flacca* and *Leucanthemum vulgare*). These included; two orchid species, *Briza media*, *Hypericum perforatum* (not an MG5 species), *Lathyrus pratensis* (present at the source at a frequency of 8%) and *Vicia cracca* (42%). The mean percentage frequency in the source of the 15 species that did not transfer was 22.55%.

Thirteen other species that had not been recorded before were found in the receiver meadow after treatment, but these were also absent from the source meadow (Table 5.4c). These 13 species were recorded only at low frequencies, the highest being *Rumex crispus* (10%; undesirable). One was a desirable species, five were neutral and seven were undesirable; three were tree seedlings. The species that were found in the receiver meadow after treatment were not necessarily found in or exclusively in the hayed

Table 5.5: Transferred sp (only) and all treatments (g – grazed, no g and bold red text- not grazed)

| **Transferred species** | Percentage frequency (9 quadrats per treatment) | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Source | Receiver 2012 | | | | | | Receiver 2013 | | | | | |
| | | **no d, no h** | **l d, no h** | **h d, no h** | **no d, h** | **l d, h** | **h d, h** | **no d, no h** | **l d, no h** | **h d, no h** | **no d, h** | **l d, h** | **h d, h** |
| *Dactylorhiza fuchsii* | 56 | - | - | - | - | - | - | - | - | - | - | - | - |
| *Heracleum sphondylium* | 18 | - | - | **6(no g)** | **4(no g)** | - | **2(no g)** | - | - | **2(no g)** | - | **4(no g)** | - |
| *Leontodon hispidus* | 17 | - | - | **2(no g)** | **2(no g)** | - | - | **2(no g)** | **4(no g)** | - | **2(no g)** | - | **2(no g)** |
| *Poa pratensis* | 34 | 9(g); **2(no g)** | 15(g) | 17(g); **2(no g)** | 15(g); **2(no g)** | 17(g); **2(no g)** | 17(g); **4(no g)** | **17(no g)** | 17(g); **15(no g)** | 17(g); **15(no g)** | 17(g); **11(no g)** | 17(g); **17(no g)** | 17(g); **17(no g)** |
| *Prunella vulgaris* | 10 | 4(g) | 2(g); **4(no g)** | **2(no g)** | 6(g) | 4(g); **6(no g)** | 4(g); **6(no g)** | **2(no g)** | 4(g); **6(no g)** | 2(g); **4(no g)** | 11(g); **6(no g)** | 9(g); **7(no g)** | 4(g); **6(no g)** |
| *Ranunculus bulbosus* | 8 | - | - | - | - | - | - | - | - | - | - | - | - |
| *Stellaria graminea* | 4 | - | - | - | - | - | - | - | - | - | - | - | - |
| *Trisetum flavescens* | 30 | - | - | - | - | - | - | - | - | - | - | **2(no g)** | **4(no g)** |

(cont.)

| **Transferred species** | Percentage frequency | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Source | Receiver 2014 | | | | | | Receiver 2015 | | | | | |
| | | **no d, no h** | **l d, no h** | **h d, no h** | **no d, h** | **l d, h** | **h d, h** | **no d, no h** | **l d, no h** | **h d, no h** | **no d, h** | **l d, h** | **h d, h** |
| *Dactylorhiza fuchsii* | 56 | - | - | - | - | - | - | - | - | - | - | - | 2(g) |
| *Heracleum sphondylium* | 18 | - | **2(no g)** | **2(no g)** | - | **2(no g)** | - | - | - | - | - | - | **2(no g)** |
| *Leontodon hispidus* | 17 | **2(no g)** | **6(no g)** | - | **7(no g)** | - | - | - | **2(no g)** | **2(no g)** | - | - | **2(no g)** |
| *Poa pratensis* | 34 | 11(g); **15(no g)** | 11(g); **11(no g)** | 9(g); **13(no g)** | 11(g); **17(no g)** | 11(g); **7(no g)** | 17(g); **15(no g)** | 17(g); **55(no g)** | 17(g); **15(no g)** | 17(g); **11(no g)** | 17(g); **15(no g)** | 17(g); **17(no g)** | 17(g); **15(no g)** |
| *Prunella vulgaris* | 10 | 4(g); **2(no g)** | 6(g); **6(no g)** | 6(g); **6(no g)** | 9(g); **13(no g)** | 9(g); **15(no g)** | 15(g); **9(no g)** | 2(g); **9(no g)** | 11(g); **7(no g)** | 11(g); **17(no g)** | 7(g); **13(no g)** | 13(g); **15(no g)** | 11(g); **15(no g)** |
| *Ranunculus bulbosus* | 8 | **2(no g)** | - | - | - | - | - | **2(no g)** | **2(no g)** | 2(g); **6(no g)** | **2(no g)** | **4(no g)** | **4(no g)** |
| *Stellaria graminea* | 4 | - | - | - | - | - | - | - | - | - | - | 2(g) | - |
| *Trisetum flavescens* | 30 | - | **2(no g)** | - | - | **4(no g)** | **2(no g)** | - | - | - | - | - | - |

Table 5.6: Number of transferred species in the individual treatments*

| Treatment | Number of additional species | | | |
|---|---|---|---|---|
| | 2012 | 2013 | 2014 | 2015 |
| No d, no h, no g | 1 | 3 | 4 | 3 |
| No d, no h, g | 2 | 0 | 2 | 2 |
| Low d, no h, no g | 1 | 3 | 5 | 4 |
| Low d, no h, g | 2 | 2 | 2 | 2 |
| High d, no h, no g | 4 | 3 | 3 | 4 |
| High d, no h, g | 1 | 2 | 2 | 2 |
| No d, h, no g | 3 | 3 | 3 | 3 |
| No d, h, no g | 2 | 2 | 2 | 2 |
| Low d, h, no g | 2 | 4 | 4 | 3 |
| Low d, h, g | 2 | 2 | 2 | 3 |
| High d, h, no g | 3 | 4 | 3 | 5 |
| High d, h. g | 2 | 2 | 2 | 3 |

* Some species are present in the more than one treatment (see Table 5.5)

strips. *C. pratensis* initially occurred at a higher frequency in hayed and then equally in both treatments. *H. mollis* was only found in hayed (and not-grazed). *V. sativa* was first only found in not-hayed and then only found in hayed, then in both and then neither (and only in not-grazed).

Nine species present in the source and in the receiver before treatment subsequently increased substantially (Table 5.4d). These species were: *Agrostis capillaris, Bromus hordeaceus, Centaurea nigra, Cerastium fontanum, Hypochaeris radicata* (generally higher in hayed (Table 5.5 and Table 5.1.1, Appendix 5.1), *Scorzoneroides autumnalis* (first recorded in hayed)*, Plantago lanceolata* (generally higher in hayed)*, Ranunculus acris* and *Trifolium pratense* (generally higher in hayed) – all desirable species. *Ranunculus acris* and *Trifolium pratense* are on the list of poor-performing species in Table 1.1, Chapter 1. The mean percentage frequency in the source of the species that increased substantially after treatment was 57.56%.

Six species present in the receiver before treatment decreased afterwards (Table 5.5e). These were: *Euphrasia* sp., *Festuca rubra* agg*.* (generally higher in hayed)*, Rhinanthus minor*, *Trifolium repens, Phleum pratense* and *Ranunculus repens*. The last two species were not in the source meadow. All except *R. repens* are desirable. The mean percentage frequency in the source of the species that decreased substantially after treatment was 50.57%. The mean percentage frequency for the species that did not change in the receiver meadow was 35.67%.

**5.3.1.3 Comparison of species following disturbance**

Of the species transferred by this experiment (Table 5.4a), *Dactylorhiza fuchsii* was only found in high-disturbance, *Heracleum sphondylium* was found most consistently in high-disturbance and *Ranunculus bulbosus* was first found in no-disturbance and then at the highest percentage frequency in high-disturbance (Appendix 5.1). *Stellaria graminea* was only found in low-disturbance (in 2015). *Trisetum flavescens* was not found in no-disturbance.

Of the species that were absent in the source and in the baseline receiver but appeared subsequently in the receiver (Table 5.4c), *Agrostis stolonifera* was only found in no-disturbance; *Alopecurus geniculatus* was first found in low-disturbance; *Arrhenatherum elatius* was only found in high-disturbance and *Betula* sp. was first found in high-disturbance, but then only in low-disturbance the following years. *Cardamine pratensis* was found at its highest frequency in low-disturbance in three out of four years. *Corylus avellana, Holcus mollis*, *Juncus effusus* and *Prunus spinosa* were only found in one year, in high-disturbance. *Rumex crispus* was found at its lowest frequencies in no-disturbance.

Of the species that increased after treatment (Table 5.4d), in the first year, *Bromus hordeaceus* was found at lowest frequency in no-disturbance and at similar frequencies in the other disturbance treatments. *Bellis perennis*, *Centaurea nigra*, *Hypochaeris radicata*, *Myosotis arvensis*, *Plantago lanceolata* and *Trifolium pratense* were generally found at higher frequencies in low-disturbance and high-disturbance.

Of the species that decreased substantially after treatment (Table 5.4e), *Lotus corniculatus* was not found for two years and then appeared only in no-disturbance; however, the following year it was found only in high-disturbance. *Rhinanthus minor* was generally found at its lowest frequency in high-disturbance and at similar frequencies in the other disturbance treatments.

#### 5.3.1.4 Comparison of species in grazed and not-grazed areas

Of the species transferred by this experiment (Table 5.4a), *Heracleum sphondylium*, *Leontodon hispidus* and *Trisetum flavescens* were found only in not-grazed (Appendix 5.1). *Ranunculus bulbosus* was first found in both treatments (in 2013), but was subsequently found only in not-grazed (2014) and then at a much higher frequency in not-grazed (2015). *Dactylorhiza fuchsii* was found only in grazed.

Of the species that were absent in the source and in the baseline receiver but appeared subsequently in the receiver (Table 5.4c), *Agrostis stolonifera* (in 2012), *Arrhenatherum elatius, Corylus avellana, Holcus mollis, Prunus spinosa*, *Vicia sativa* and *Vicia sepium* were found only in not-grazed. *Rumex crispus* was found at much higher frequencies in grazed. Bare ground was only found in grazed.

Of the species that increased after treatment (Table 5.4d), *Agrostis capillaris* was found only in not-grazed in 2012 and at a much higher frequency in not-grazed than grazed in subsequent years. *Plantago lanceolata* was found at similar levels in both treatments in 2012, but after this was found at higher levels in not-grazed. *Ranunculus acris* was found at slightly higher levels in not-grazed in 2012 and 2013. *Scorzoneroides autumnalis* was found only in not-grazed in 2012, was then found in both at similar levels in 2013 and 2014 and then at higher levels in grazed in 2015. *Bellis perennis* was found only in grazed in 2012 and in subsequent years was found at higher frequency in grazed than not-grazed. *Cerastium fontanum* was found at a higher frequency in grazed than not-grazed, in 2012. *Myosotis arvensis* was first found only in grazed (2012) and then in both treatments. *Trifolium* *pratense* was found at higher frequencies in grazed, in three out of four years.

Of the species that decreased substantially after treatment (Table 5.4e), *Euphrasia* sp. was found only in grazed in 2012 and 2013 and then at a higher frequency in not-grazed, in 2014 and 2015. *Rhinanthus minor* was found at similar levels in both treatments for two years and was then found at much higher levels in not grazed for two years. *Lotus corniculatus* was first found only in not-grazed (2014) and was then found in both treatments.

Of the species that did not change in frequency substantially overall (Table 5.4f), *Taraxacum* sp. was found at higher frequencies in not grazed in three out of four years. *Trifolium dubium* was found at a much higher frequency in grazed in 2012 and then at similar frequencies in both treatments in subsequent years.

#### 5.3.1.5 Comparing combined treatment effects on transferred species

The eight species that were additional species after treatment were found in varying treatment blocks (Tables 5.5, 5.6). That is, some species were found in all or most treatments in all or most years (*Poa pratensis, Prunella vulgaris*; Table 5.5). Other species were found in some treatments in some years, but not in others. In every year, additional species were found in most treatments (the exception being no-disturbance/ not-hayed/ grazed, in 2013; Table 5.6). In most cases, the number of additional species was higher in the not-grazed counterpart of every treatment in every year - the exceptions being no-disturbance/not-hayed and low-disturbance/not-hayed, both in 2012 and low-disturbance/ hayed in 2012 and 2015, in which numbers were equal (Table 5.6).

*Dactylorhiza fuchsii* was only found in quadrats in 2015, in high-disturbance/ hayed/grazed quadrats (Table 5.5). *Stellaria graminea* was also only found in 2015, in only one treatment type, that of low-disturbance/hayed/grazed. All the other species that were additional post treatment were found in several different treatments and some were also recorded in different treatments in different years. For example, *Heracleum sphondylium* was found in a number of different treatments including hayed and not-hayed and in all levels of disturbance treatments, in different years. However, it was never found in grazed quadrats. *Prunella vulgaris* was found in all treatment types in all years, except for no-disturbance/ not-hayed/ not-grazed; no-disturbance/hayed/not-grazed and high-disturbance/not-hayed/ grazed, in 2012 and no disturbance/not-hayed/grazed in 2013. Where it was found in both the grazed and not-grazed areas of a treatment, in 13 out of 20 cases, it was found at a higher percentage frequency in the not-grazed area. *Poa pratensis* shows the opposite pattern, being more often found at higher percentage frequencies in the grazed area of a treatment.

*Ranunculus bulbosus* was first found, in 2014, only in no-disturbance/not-hayed/not-grazed, but in the following year was found in all combinations of haying and disturbance, although only once in a grazed area (high-disturbance/not-hayed/grazed). *Trisetum flavescens* was first found in low-disturbance/hayed/not-grazed and high-disturbance/hayed/not-grazed, in 2013. It was also found in these two treatments in 2014, plus low-disturbance/not-hayed/not-grazed.

### 5.3.1.6 Significance testing of treatment effects

The factorial repeated measures ANOVA conducted to determine the effects of year (4), haying (2), grazing (2) and disturbance (3) levels on the mean percentage cover of the desirable species showed a statistically significant three-way interaction between haying, grazing and disturbance [$F(2,56) = 3.505$, $p = 0.037$], but no other significant three-way interaction. Hayed/not-grazed/high-disturbance had the highest mean percentage cover (0.38 (95% CI, 0.12 to 1.22) of desirable species and not-hayed/grazed/no-disturbance had the lowest 0.14 (95% CI, 0.04 to 0.55). In descending order, the other means for treatment combinations were: not-hayed/not-grazed/low-disturbance 0.32 (95% CI, 0.11 to 0.94); hayed/not-grazed/low-disturbance 0.27 (95% CI, 0.08 to 0.95); hayed/grazed/low-disturbance 0.25 (95% CI, 0.07 to 0.85); hayed/not-grazed/no-disturbance 0.23 (95% CI, 0.07 to 0.77); not-hayed/not-grazed/high-disturbance 0.22 (95% CI, 0.06 to 0.74); hayed/grazed/no-disturbance 0.21 (95% CI, 0.06 to 0.74); not-hayed/grazed/low-disturbance 0.20 (95% CI, 0.06 to 0.72); not-hayed/not-grazed/no-disturbance 0.20 (95% CI, 0.06 to 0.70); not-hayed/grazed/high-disturbance 0.20 (95% CI, 0.06 to 0.68) and hayed/grazed/high-disturbance 0.19 (95% CI, 0.05 to 0.69).

There was a statistically significant two-way interaction between year and disturbance for not-grazed, [$F(5.167,144.665) = 2.838$, $p = 0.017$, $\varepsilon = 0.861$ (Huynh-Feldt)] and also between year and haying [$F(3,84) = 2.769$, $p = 0.047$]. There was a statistically significant two-way interaction between year and disturbance for grazing, [$F(5.225,146.306) = 2.309$, $p = 0.045$, $\varepsilon = 0.871$ (Huynh-Feldt)].

Following the initial three-way interaction, there was a statistically significant two-way interaction between year and disturbance for not-hayed, [$F(6,168) = 3.067$, $p = 0.007$] and between grazing and disturbance for haying [$F(1.669,46.734) = 3.468$, $p = 0.047$, $\varepsilon = 0.799$ (Huynh-Feldt)], but no statistically significant interaction or simple main effect of disturbance at either level of grazing for the hayed treatments. However, there was a statistically significant two-way interaction between year and grazing for the intermediate level of disturbance [$F(3,84) = 3.466$, $p = 0.020$] in the hayed treatments, although not for the high or low levels.

Following the initial three-way interaction, there was a statistically significant two-way interaction between year and grazing for the intermediate level of disturbance [$F(2.367,66.267) = 5.107$, $p = 0.006$, $\varepsilon = 0.789$ (Huynh-Feldt)] but not for the high or low levels. There were no further significant effects.

Sphericity was assumed for all these tests, as assessed by Mauchly's Sphericity Test, unless otherwise stated. The mean values are low due to a high number of zeros in the data.

### 5.3.2 Summary of the main results

- 23 species were present in the source but not in the baseline receiver, 19 of which were desirable. 7 were on the list of poor performing MG5 species in Chapter 1, plus 3 orchid species.
- 8 species were transferred (by 2015), 7 of which were desirable, 1 on the list of poor performing MG5 species and 1 orchid.
- 12 desirable species did not transfer (8 of which are MG5 species, constancy I-IV) and 3 other species (2 of which are MG5 species). 6 were on the list of poor performing MG5 species, plus 2 orchid species.
- 12 species increased in the receiver after treatment, 9 of which were in the source meadow: 10 were desirable species and 10 were MG5 species. 2 were on the list of poor performing MG5 species.
- 7 species decreased in the receiver after treatment, all of which were in the source meadow: 6 were desirable and 5 were MG5 species.
- When considering the combinations of treatments, the transferred species were present in varied treatments: some in all treatments and all/most treatments had some transferred species present. Species were also found in some treatments in some years, but not in others.

- When considering the treatments in isolation, *Stellaria graminea* was only found in a hayed area, *Trisetum flavescens* was first found in hayed and was then found at higher percentage frequency in the not-hayed area.
- *Dactylorhiza fuchsii* was only found in high-disturbance, *Heracleum sphondylium* was found most consistently in high-disturbance and *Ranunculus bulbosus* was first found in no-disturbance and then at the highest percentage frequency in high-disturbance. *Stellaria graminea* was only found in low-disturbance. *Trisetum flavescens* was not found in no-disturbance.
- *Heracleum sphondylium*, *Leontodon hispidus* and *Trisetum flavescens* were found only in not-grazed. *Ranunculus bulbosus* was first found in both treatments, but was subsequently found only in not-grazed and then at a much higher frequency in not-grazed. *Dactylorhiza fuchsii* was found only in grazed.
- A factorial repeated measures ANOVA showed that there was a statistically significant three-way interaction between haying, grazing and disturbance on the mean percentage cover of the desirable species. Hayed/ not-grazed/ high-disturbance had the highest mean percentage cover of desirable species and not-hayed/ grazed/ no-disturbance had the lowest. Of these treatment combinations, those with haying tended to have higher means than those without haying, although there were no main significant effects for any of the treatments. Conversely, the presence of grazing was associated with lower means. The effect of different levels of disturbance was much more complex and most follow-up interactions involved year.

- There were statistically significant two-way interactions between year and disturbance for not-hayed and hayed and between year and grazing for the intermediate level of disturbance in the hayed treatments.
- There was also a statistically significant two-way interaction between year and grazing for the intermediate level of disturbance.

## 5.4 Discussion

### 5.4.1 Comparison between the source and the receiver meadow before treatment

Twenty-three species that were present in the source meadow were absent from the baseline receiver, over half of the species present in the source, suggesting that the two meadows were very different. PCA and MAVIS analyses also suggest that the source meadow and the baseline receiver were not similar (Appendices 5.4, 5.5).

### 5.4.2 Introduction of additional species

The percentage frequencies in the source meadow of the species that did transfer appear to be generally higher (4-100%; mean: 33%) than those of the species that did not (walkover-58%; mean 23%).  Four of the eight species (*Heracleum sphondylium, Leontodon hispidus, Poa pratensis* and *Prunella vulgaris*) established in the first year after strewing and were recorded in every post-treatment year. The first two species were recorded at lower percentage frequencies than at the source in every year and the latter two were recorded at higher percentage frequencies in every year. This may be due to the conditions at the receiver being more (in the latter case) or less (in the former case) favourable than at the source. These conditions could include: soil moisture levels, soil bacteria: fungi ratio, competition or inhibition from established vegetation, lack of facilitator species and/or management differences (Grime *et al.,* 1988; Bullock *et al.,* 2001; Smith *et al.,* 2003; Smith *et al.,* 2008; Dunn and Tallowin, 2012).

Species being recorded at a lower percentage frequency than at the source could be due to the time taken for the population to increase from establishment, or that conditions are not favourable in the receiver. All of these species were recorded in both hayed and not-hayed areas, although *H. sphondylium* disappeared from not-hayed in 2015 and *P. vulgaris* was recorded at substantially higher frequencies in hayed. *Poa pratensis* was found in significantly higher amounts in the high-disturbance treatment.

*H. sphondylium* is a CR strategist, associated with a middle ranking floristic diversity (14.1-18.0 species per metre squared; Grime *et al.,* 1988). *P. vulgaris* is a CSR strategist with an associated floristic diversity of over 22 species per metre squared (Grime *et al.,* 1988). Trueman and Millett (2003) report a 50% success rate with both these species, Smith *et al.* (2000) found infrequent establishment of *L. hispidus*. *Leontodon hispidus* is a stress-tolerator associated with relatively undisturbed and infertile habitats and relatively high floristic diversity (Grime *et al.,* 1988). *Poa pratensis* is a CSR strategist with a middle ranking floristic diversity (Grime *et al.,* 1988). *L. hispidus, P. pratensis* and *P. vulgaris* all have small seeds (Grime *et al.,* 1988), which could have either blown into the not-hayed area or stuck to the machinery and been transferred in this way during the strewing. *H. sphondylium* has larger, heavier seeds, but they are adapted for dispersal by wind, which could explain their appearance in the not-hayed areas (Grime *et al.,* 1988).

*Trisetum flavescens* was the only additional species recorded in 2013; it was only observed in hayed areas. This species was also recorded in 2014 (in hayed and not-hayed, although at a much higher level in hayed), but not in 2015. Its percentage frequency at the receiver was a tenth of that at the source. This is a similar result to Trueman and Millett (2003). *Trisetum flavescens* is a CSR strategist, associated with meadows and habitats with bare soil and with a relatively high floristic diversity (Grime *et al.,* 1988). Lencova and Prach (2011) found that this was a typical species of early and middle-succession (restoration *sensu* Dunn and Tallowin (2012)) stages, when restoring hay meadows on ex-arable land (with seed mixtures or spontaneous succession).

*Ranunculus bulbosus* was the only additional species recorded in 2014 and it was recorded at a lower percentage frequency than at the source. It was also recorded in 2015, at a slightly higher percentage frequency than at the source. In 2014 it was only recorded in not-hayed/no-disturbance/not-grazed and in 2015 it was recorded in hayed and not-hayed, at similar levels. *R. bulbosus* is intermediate between a SR and a CSR strategist and is associated with a high floristic diversity (Grime *et al.,* 1988). It has a relatively high seed weight, but is a common species of grasslands (Grime *et al.,* 1988).

The remaining species transferred by this experiment were first recorded in 2015: *Dactylorhiza fuchsii* and *Stellaria graminea*, both at lower frequencies than at the source. *D. fuchsii* was recorded in hayed and not-hayed and *S. graminea* was only recorded in hayed. Orchids are thought to take a number of years to establish after sowing (Trueman and Millett, 2003; Kotilinek *et al.,* 2015). Therefore, this species could have established due to the previous green hay strewing. It could also be that the mycorrhizae were present and well established from the previous strewing. *D. fuchsii* has dust-like seed, which may have been blown into the not-hayed areas, either from this or the previous hay strewing. It is intermediate between a stress-tolerator and a CSR strategist and has a very high associated floristic diversity (Grime *et al.,* 1988). Green hay strewing is known to be an effective technique for transferring orchids, as it is thought to include the transfer of the relevant mycorrhizae (Trueman and Millett, 2003). *Stellaria graminea* is a CSR strategist, with a relatively high associated floristic diversity and relatively small seeds (Grime *et al.,* 1988).

Of the species that established in the receiver meadow after treatment, but were absent from the source, only one was desirable, *Cardamine pratensis*, and it was recorded in every post-treatment year, in hayed and not-hayed quadrats. This is potentially due to a change in conditions in the receiver meadow (before to after treatment) and, as this species has a preference for damp conditions (Grime *et al.,* 1988), may be due to a series of wetter years (Meteorological Office, 2015). Several other species absent from the baseline receiver and the source, but present in the receiver meadow after treatment, are also associated with damp/wet conditions, suggesting that the conditions in the receiver meadow have become wetter during the experiment, compared to the baseline receiver.

### 5.4.3 Species that did not transfer

Of the species that did not transfer, *Conopodium majus* had the highest percentage frequency (58%) in the source meadow. As mentioned in Chapter 4, this species flowers relatively early (May-June), with the shoot being dead by July (Grime *et al.,* 1988), which may mean that the seed was shed/lost before the hay was cut. However, Trueman and Millett (2003) report success in four out of six attempts, although only at low levels in the receiver meadow.

Three species with source frequencies of around 40% did not transfer: *Dactylis glomerata* (40%), *Vicia cracca* (42%) and *Anacamptis morio* (38%). It may be surprising that *D. glomerata* and *V. cracca* did not transfer, as they are generalist, competitive species (Grime *et al.,* 1988). *V. cracca* has late seed set, shedding seed from August (Grime *et al.,* 1988), which may explain why it did not transfer. As mentioned in previous chapters and in relation to *D. fuchsii* above, orchids can take time to establish after transfer

(Trueman and Millett, 2003), which may explain why *A. morio* has not yet been recorded in the receiver meadow. Conversely, Trueman and Millett (2003) reported success with *D. glomerata* and *V. cracca* in five out of six and four out of six attempts, respectively.

*Achillea millefolium* (32%) and *Briza media* (20%) also had relatively high percentage frequencies in the source meadow. *A. millefolium* sets seed from July (Grime *et al.,* 1988), which may have been after the time of the green hay cut and the reason why this species did not transfer. *Briza media* also has a July seed set date (Grime *et al.,* 1988). *A. millefolium* is often unsuccessfully transferred, whereas transfer of *B. media* is normally successful, although it does not establish at high frequencies (Trueman and Millett, 2003). Smith *et al.* (2003) also failed to establish *Briza media* from seed sown when aiming to enhance MG6 *Lolium perenne-Cynosurus cristatus* grassland. Other studies have found success in establishing *A. millefolium* from seed (e.g. Gilbert, 1991).

The remaining species that did not transfer all had frequencies in the source meadow of <8%. *C. flacca* is known to be difficult as sedges spread more by vegetative means than by seed and prefer wet ground (Grime *et al.,* 1988). Trueman and Millett (2003) report success in three out of six attempts with *L. pratensis*. *Ajuga reptans* also prefers wet ground and is also a relatively early flowering species (May; Grime *et al.,* 1988) *Primula veris* is another early flowering species (April; Grime *et al.,* 1988) and is also low growing, which could mean that seeds are missed by the hay cutting and baling machinery (Scotton *et al.,* 2009), although it has transferred in other experiments, e.g. four successes in six attempts (Trueman and Millet, 2003). *Polygala vulgaris* is another low growing species and it has very small seeds (Grime *et al.,* 1988). *Potentilla erecta* was observed as having a low growing habit at Three Yew Trees and also has small seeds (Grime *et al.,* 1988). *Prunella vulgaris* was successfully transferred and is low-growing, but was observed as tall in the source meadow.

*Neottia ovata* is another orchid species and, as mentioned above, orchids are known to take time to establish in created meadows (Trueman and Millett, 2003), which may explain why it has not yet established. *Hypericum perforatum* and *Leucanthemum vulgare* were recorded at only very low frequencies in the source meadow, which may explain why they have not transferred. *Leucanthemum vulgare* is normally very easy to transfer (Besenyei, 2000; Trueman and Millett, 2003; Rayner, 2005).

### 5.4.4 Changes in frequencies of existing species

The species present in the baseline receiver that increased substantially after treatment had the highest percentage frequency (58%) in the source meadow of the five groups of species (i.e. species that did transfer, species that did not transfer, species that increased in frequency in the receiver, species that decreased and species that did not change; Table 5.4), although three were not present in the source meadow. Ten species were desirable, one was neutral and one, *Rumex obtusifolius* (absent from the source), was undesirable; 10 were MG5 species. They are all relatively generalist species even though they are associated with habitats of high species richness (Grime *et al.,* 1988).

The species that were present in the baseline receiver that decreased substantially after treatment had a mean percentage frequency in the source meadow that was the second highest of the five groups. Only one of these species was not in the source meadow.  All but one are desirable and all but two are MG5 species. They included *Lotus corniculatus,* which has been found to be difficult to establish in previous studies (Hopkins *et al.,* 1999; Smith *et al.,* 2000; Besenyei, 2000; Hofmann and Isslestein, 2004; Rayner, 2005 and Chapter 4, see Section 4.4.4).

Changing conditions in the receiver meadow may have led to a reduction in these species and those present in the source may also not have transferred. For example, if the soil moisture levels increased due to the wetter weather. *Quercus* sp. would not have been expected to transfer, as it was a small sapling that would not have been producing seed. *Euphrasia* sp., *Festuca rubra, Rhinanthus minor* and *Trifolium repens* were all recorded in the baseline receiver at high percentage frequencies. *Euphrasia* sp. had a particularly dramatic reduction in frequency (70% in 2011 to 1% in 2012). There is no obvious reason for these species to decline, but possibilities could include: the weather, damage by the experimental process, a bad year for this species or natural fluctuations in the population. *Lotus corniculatus,* *Luzula campestris* and *Quercus* sp. (an undesirable species) were all recorded at only low percentage frequencies in the baseline receiver, so may have been missed in subsequent years (the former two species reappear at similar frequencies in later years).

As mentioned in Section 4.4.4, *L. corniculatus* can be a low growing species, with just a moderate number of large seeds produced and only infrequently (Grime *et al.,* 1988). The large seed size may mean that the seeds drop out of the hay before transfer. Other studies report success with this species (e.g. Trueman and Millett, 2003). *L. campestris* is intermediate between a stress-tolerator and a CSR strategist and flowers early, but does not shed seed until July and August (Grime *et al.,* 1988). It is a relatively low-growing species, which may explain its lack of transfer. Losvik and Austad (2002) were not successful in establishing *L. campestris,* by contrast, Trueman and Millett (2003) report success in six out of six attempts.

### 5.4.5 Comparing combined treatment effects

Statistical analysis showed a significant interaction between haying, grazing and disturbance treatments in their effect on the mean percentage cover of the desirable species. Hayed/not-grazed/high-disturbance had the highest mean percentage cover of desirable species and not-hayed/grazed/no-disturbance had the lowest. Subsequent two-way interactions mostly involved year, but the absence of subsidiary main effects made it difficult to interpret these effects apart. This suggests that hayed/not-grazed/high-disturbance was the best treatment combination to encourage desirable species. Statistical analysis on just introduced target species (i.e. those present in the source but not in the receiver after treatment) could not be carried out as there were only a small number of records of these species.

For the eight individual species that were additional after treatment, there was no pattern in their occurrence in the combinations of treatments, as they were found in varying treatment blocks.

### 5.4.6 Differences between years

As in previous chapters, statistical testing showed complex interactions between year and the treatment variables which could be due to several reasons, including: the effect of weather or other changing conditions from year to year (e.g. the effect of liming, in this field), changes in the vegetation due to its development over the years (i.e. the haying taking several years to take effect) or differences between quadrat locations from year to year. In terms of the differences in species between the years, once a species had been introduced, five out of eight (*H. sphondylium, L. hispidus, P. pratensis, P. vulgaris* and *R. bulbosus*) were found in all subsequent years. *D. fuchsii* and *S. graminea* were only found in the last year of quadrat surveys. *T. flavescens* was recorded in 2013 and 2014, but not 2015. PCA indicates differences between the years (Appendix 5.5).

### 5.4.7 Differences between hayed and not-hayed treatment areas

Of the species that were introduced by this experiment, *Stellaria graminea* was the only introduced species found only in the hayed area. *Trisetum* *flavescens* was first found only in hayed but was then found in both. *Ranunculus bulbosus* was first found in not-hayed but was then found in both and the other five species were all found in hayed and not-hayed at the same time. Species being found in both treatments could be due to them being missed in the baseline survey, to seeds sticking to machinery or footwear and being shed in not-hayed areas or being blown into not-hayed areas. In later years, it could also be due to management activities leading to hay being spread from hayed areas into not-hayed areas and *vice versa.*

Of the species that increased post-treatment, *Hypochaeris radicata, Plantago lanceolata* and *Trifolium pratense* had higher amounts in hayed, whereas the remaining nine species had relatively equal amounts in hayed and not-hayed.

### 5.4.8 Differences between disturbance treatments

The introduced species appear to have responded differently to disturbance. This could be due to a requirement for large gaps in the existing vegetation, for species that were only (or mostly) found in high-disturbance or a requirement for a gap with cover from existing vegetation, for species found only (or mostly) in low-disturbance, although statistical analysis suggested that there was little difference between the two treatments. As the area was already managed as a meadow, with a hay cut and aftermath and spring-grazing, it can be assumed that there were some gaps already in the existing vegetation, which may explain why some species established in the no-disturbance areas. Donath *et al.* (2007) found that rotavation had only a small positive effect on establishment on grassland sites, whereas Edwards *et al.*, (2007) found a positive effect of power harrowing when enhancing lowland hay meadows, in conjunction with seed addition. Besenyei (2000) found that the effect depended on the pre-existing vegetation and had a negative effect where pernicious weeds were present.

Disturbance appeared to have a positive effect on some species that were already present in the receiver meadow. This could be through the creation of suitable regeneration gaps for either seeds of existing plants or seeds of these species introduced in the hay. However, other species appeared to be negatively affected by disturbance.  A negative effect could be due to, for example, damage to existing plants or burying of the seeds combined with a lack of establishment of new plants from the hay.

Disturbance can also potentially create gaps for undesirable species to colonize. In this experiment four undesirable and two neutral species were associated with the high-disturbance treatment, although three of these species also have a preference for damp/wet soil conditions (*Alopecurus geniculatus*, *Holcus mollis* and *Juncus effusus;* Grime *et al.,* 1988), which may explain their appearance in the meadow, as a consequence of wetter conditions created by a series of wet summers (Meteorological Office, 2015). Two of the undesirable species were tree species, possibly from the hedgerow which surrounds the meadow. These should be eliminated through hay meadow management; *Arrhenatherum elatius* should also be controlled by management. *Rumex crispus* was also associated with disturbance (both low and high) and may need pulling/weed treatment to control it. This suggests that disturbance is not problematic where weed species are under control and proper management is in place.

### 5.4.9 Differences between grazing treatments

The introduced species appear to have responded differently to grazing, as has been found previously (Crofts and Grayson, 1999; Bullock *et al.,* 2001; del-Val and Crawley, 2005; Critchley *et al.,* 2007; Rinella and Hileman, 2009). This could be due to positive effects of grazing, such as gap creation and control of vegetation height or negative effects such as selective grazing of particular desirable species or grazing of seedlings, especially of the new species (Bullock *et al.,* 2001). Four of the eight species that were transferred appear to be associated with the not-grazed treatment, suggesting that their seedlings may have been grazed off in the grazed treatment. Grazing would be expected to favour grasses and low-growing/rosette-forming species, but this is not obvious in this study, possibly because the sheep have favoured grazing these species.

Grazing treatment would not be expected to negatively affect the existing species in the meadow as spring- and autumn-grazing was part of the normal management of this meadow before the experiment was implemented. However, the species that were missing prior to treatment, may have been missing due to the existing management regime, therefore, these species may have been introduced in the hay, but have then been removed again by the grazing regime. Robertson and Jefferson (1999) state that the effect of spring grazing on the species composition of MG5 grasslands is not well understood. Smith *et al.* (2000) found that spring grazing, autumn grazing, seed addition and 21st July cut date resulted in the highest species diversity cf. other combinations, four years after creation. However, advice to agri-environment and other conservation schemes was for a mid-July cut date and autumn grazing (Smith *et al.,* 2008). Critchley *et al.* (2007) found a detrimental effect of spring-grazing when it was prolonged. Smith *et al.* (1996) found that there was more *Trisetum flavescens* with autumn-grazing and more *Avenula pubescens* and *Ranunculus repens* with autumn- and spring-grazing when comparing no grazing, autumn-grazing and spring- and autumn-grazing treatments. DEFRA (2012) found that *Rhinanthus minor*, *Conopodium majus*, *Ranunculus bulbosus*, *Anemone nemorosa*, *Festuca rubra* and *Anthoxanthum odoratum* were associated with early shut dates in MG3 grasslands and *Anthoxanthum odoratum*, *Conopodium majus* and *Ranunculus bulbosus* were associated with early shut dates in MG6 grasslands. For *R. minor, C. majus* and *T. pratense*, plus two other species, the general pattern with later shut dates was of delayed flowering and less seed set. The experiment overall found that spring-grazing reduced plant diversity in northern upland meadows and suggests that the maintenance of these meadows would be best supported by low-intensity grazing and early shut dates.

It should also be noted that a grazing treatment was not part of the original plan for this experiment and the layout of this treatment was not ideal as it corresponded to a slope on the site: i.e. the not grazed area was at the bottom of the slope and the grazed area was at the top of the slope. This means that the grazing treatment effects could in fact be due to the slope and not due to the treatment. The layout was chosen so that either the grazed or not grazed quadrats could be disregarded, if necessary, without loss of replications.

## 5.5 Conclusions

The species that were missing from Golden Field compared to Three Yew Trees were: *Dactylorhiza fuchsii, Heracleum sphondylium, Leontodon hispidus, Poa pratensis, Prunella vulgaris, Ranunculus bulbosus, Stellaria graminea, Trisetum flavescens, Achillea millefolium, Ajuga reptans, Anacamptis morio, Briza media, Carex flacca, Conopodium majus, Dactylis glomerata, Hypericum perforatum, Lathyrus pratensis, Leucanthemum vulgare, Neottia ovata, Polygala vulgaris, Potentilla erecta, Primula veris,* and *Vicia cracca.* Of these, *Briza media, Carex flacca, Conopodium majus, Dactylis glomerata, Heracleum sphondylium, Lathyrus pratensis* and *Vicia cracca* are on the list of poor performing MG5 species from a review of the literature (Chapter 1, Table 1.1). Orchids are also identified in the literature as difficult species to establish in created meadows. The first eight species on this list were transferred by this experiment (all desirable, except for *Stellaria graminea,* which was neutral and all MG5 species except for *Stellaria* *graminea* and *Dactylorhiza fuchsii,* although both are meadow species). The remaining 15 species were not transferred (12 desirable and three neutral MG5 species).

This experiment found that green hay strewing increased the number of species in an existing species-rich meadow and can also increase the frequency and abundance of existing species. Overall, species that did transfer had a higher percentage frequency in the source meadow than the species that did not transfer, although some individual species of low frequency did transfer and some individual species of high frequency did not. This suggests that strewing green hay onto an existing species-rich meadow is a viable technique to introduce missing species, although some individual species may need other techniques. The significant three-way interaction between haying, grazing and disturbance shows that the relationships are complex. Hayed/not-grazed/high-disturbance had the highest mean percentage cover of desirable species and not-hayed/grazed/no-disturbance had the lowest. There were also statistically significant two-way interactions involving year in some of the treatments.

# Chapter 6

# Axenic seed germination and *in vitro* propagation of the meadow orchid species *Dactylorhiza fuchsii* (Druce) Soo

## 6.1 Introduction

A number of orchid species are associated with species-rich meadows (Rodwell, 1992). Orchids are generally viewed as one of the more difficult families to establish in created, restored or enhanced meadows, although some success has been achieved with hay strewing (Trueman and Millett, 2003; Natural England, 2010a). Orchids may also take longer to appear in a new community than other species (Trueman and Millett, 2003), thus, most monitoring schemes may be published too soon, before these species become established. Consequently, there are several suggestions in the literature about the need for longer term studies (e.g. Turnbull *et al*., 2000; Baasch *et al*., 2010). In spite of this, meadow creation and restoration projects often have orchids as target species (including agri-environment schemes in the UK (Natural England, 2010b) and the global loss of orchid habitat over the last 100 years, coupled with concerns over impacts from climate change and other human activities, has led to increased interest in orchid conservation (Stewart and Kane, 2006; Seaton *et al.,* 2010; Ashmore *et al.,* 2011; Ercole *et al*., 2013; Krupnick *et al.,* 2013; Bustam *et al.,* 2014; Merritt *et al.,* 2014; Rankin *et al.,* 2014; Pierce *et al.,* 2015).

A variety of conservation tools are being implemented, including *ex situ* and translocation techniques (Swarts and Dixon, 2009; Kew, 2013), as well as hay meadow creation through green hay strewing (Trueman and Millett, 2003). The establishment of new populations derived from propagated orchids is also considered to be an important technique (McKendrick 1995; Scade *et al*. 2006; Ashmore *et al*., 2011; Krupnick *et al*., 2013), although more work is required on seed germination methods (Stewart and Kane 2006; Malmgren, 2011) and field establishment techniques (Batty *et al*. 2006; Scade *et al*., 2006; Kew, 2013).

To date, there are published studies for only a few terrestrial orchid species (Kauth, 2010), although more work has been carried out by interested individuals and groups. For example, Malmgren describes optimal techniques for growing a range of terrestrial orchids on his website (Malmgren, 2011) and there are also several books on the subject (e.g. Seaton and Ramsay, 2005; Seaton *et al*., 2011; Barnwell, 2012; Cook, 2013). Plant suppliers, such as Thompson and Morgan, sell a limited number of British terrestrial orchid species (Thompson and Morgan, 2014), as do projects such as that based at Writhlington School (Writhlington Orchid Project, 2012). The Hardy Orchid Society (HOS) have grown some native orchid species from seed for several projects in partnership with organisations such as Plantlife and the Berkshire, Buckinghamshire and Oxfordshire Wildlife Trust (BBOWT) and have also undertaken various reintroduction projects (HOS, 2014).

Axenic (tissue culture based) orchid seed germination methods include symbiotic and asymbiotic techniques, both of which are valuable for orchid conservation (Yam and Arditti, 2009; Bustam *et al.,* 2014). These methodologies produce plants that can be kept in collections, such as those in Botanical Gardens, for study, for the production of more plants and also for introduction and re-introduction projects (e.g. Ramsay and Stewart, 1998; Sprunger and Prendergast, 2010). Symbiotic techniques involve the use of a mycorrhizal fungus to stimulate germination, whereas asymbiotic techniques involve the use of certain sugars within the media to bring about germination. This latter technique was conceived by Knudson (1922) who, after determining that it was not the fungi that stimulated germination, but the fungal products, concluded that these products could be used without the fungi needing to be present (Knudson, 1922; Knudson, 1924).

A range of media are now used for axenic orchid seed germination and new media are continually being developed and tested in order to further increase the success of germination and subsequent growth of plantlets to maturity (e.g. Ponert *et al*., 2011; Pierce *et al.,* 2015). Media include those based on oats for symbiotic techniques, with a range of additions: such as charcoal (for pH balancing) and sources of complex carbohydrates, such as swede, banana and potato, for the continuing growth of the protocorms (Seaton and Ramsay, 2005; Malmgren, 2011; Seaton *et al*., 2011). Asymbiotic media include: Knudson (B and C), PhytoTechnology P668, Murashige and Skooge (MS) and Vacin and West – different media being suitable for different species or genera (e.g. Dijk and Eck, 1995; Znaniecka and Ojkowska, 2004; Millner *et al.*, 2008; Yam and Arditti, 2009). The development of asymbiotic media (containing a source of soluble sugars, which would naturally be provided by the mycorrhizae) meant that orchid species could be grown in the laboratory without the need to isolate their specific symbiotic fungi (Yam and Arditti, 2009).

The stage of maturation of the capsule and therefore the seeds can also affect the efficacy of germination (Znaniecka and Ojkowska, 2004) and seeds within even the same capsule can be at different stages of maturity (Seaton and Ramsay, 2005). Optimal germination conditions differ between terrestrial orchid species and therefore an individual method needs to be developed for each species in order to obtain the most efficient results (Znaniecka and Ojkowska, 2004). When protocorms have developed sufficiently into plantlets, these can be transferred *ex vitro* for later planting in appropriate meadow sites. However, the high levels of humidity in *in vitro* containers mean that plants acclimatized to these conditions are difficult to transfer *ex vitro* (to compost; Ramsay and Stewart, 1998).

### 6.1.1 Studies on orchid species associated with meadows

Orchid species are only recorded as low frequency associates of MG5 *Cynosurus cristatus-Centaurea nigra* meadows in the NVC (Rodwell *et al.*, 1991 *et. seq.)* – these species are: *Orchis morio (Anacamptis morio), Listera ovata (Neottia ovata)*, *Platanthera bifolia*, *Platanthera chlorantha* and

*Coeloglossum viride* (Rodwell, 1992). *Dactylorhiza fuchsii* (Figures 6.1 and 6.2), although not listed in the NVC as an MG5 species, was chosen for this study, as it is often found in MG5 species-rich meadows in the West Midlands, including Illey Pastures SSSI, Eades Meadow NNR, Draycote Meadows SSSI and Pikes SSSI. It is therefore a target species for meadow creation and improvement projects in this region. Additionally, *Platanthera bifolia, Platanthera chlorantha* and *Coeloglossum viride* have not been recorded in Birmingham and the Black Country and *Listera ovata* and *Orchis morio* have only been recorded at sites where green hay has been strewn (Trueman *et al.,* 2013). *Orchis morio* also transfers very easily with green hay (Trueman and Millett, 2003). On the other hand, *D. fuchsii is* recorded in Birmingham and the Black Country and, as this area was likely to be the focus of future introduction projects/experiments, it made this species an appropriate choice, as it is known to survive here. van Waes (1987) tested the effect of activated charcoal on axenic propagules of 18 Western European orchids, including *Orchis morio (Anacamptis morio), Listera ovata (Neottia ovata)* and *Platanthera chlorantha* from the preceding list. For all the tested species, the addition of activated charcoal to the sowing medium resulted in lower germination rates and slower development of the resultant protocorms. Conversely, the addition of activated charcoal to the transplantation medium, stimulated growth in most cases.

*Dactylorhiza praetermissa* is also found in hay meadows and the effect of differing amounts of nitrogen and phosphorus on this species *in vitro* was studied by Dijk and Eck (1995) along with the effect on other Dutch marsh orchids. They found that only high concentrations of nitrogen (e.g. 12 mM mineral N) had a general negative effect on orchid axenic growth and that responses varied according to the species: *D. praetermissa* responded positively to addition of mineral nitrogen in the media, but negatively to the addition of phosphorus.

Znaniecka and Ojkowska (2004) compared axenic asymbiotic germination of mature and immature seeds of five endangered European orchid species in order to establish an *in vitro* technique for them. In three of the species the highest level of germination occurred when immature seeds were used, in one species germination was not successful at all. For *A. morio,* the only species tested that is associated with MG5 meadows, only mature seeds were obtained and germination was observed (Znaniecka and Ojkowska, 2004).

Jakobsone (2008) studied the development of *D. fuchsii* seedlings on asymbiotic media, a modified formula of Knudson and MS media, using half mature seeds and incubation in the dark. Plants with two leaves, at an appropriate stage for transplantation into soil, were produced after eight-nine months from the start of germination – i.e. a significantly shorter time than under natural conditions (Jakobsone, 2008). Jakobsone (2009) experimented with *D. fuchsii* and eight other species and achieved germination of seven of these species. Plants of two species (*D. fuchsii* and *Gymnadenia conopsea*) were successfully transferred *ex vitro* after two years of growth. Other work on relevant species includes that of Malmgren (1988; 1989; 1993), who has published results of his work on his website (as previously mentioned) and McKendrick (1995), who studied field transplanted laboratory-raised *D. praetermissa* in southern England.

Other 'difficult' species are also being studied in this way to improve understanding of the germination/ establishment requirements of the seeds and to improve field establishment techniques for the propagated plants. For example, the UK Native Seed Hub at Kew is studying a range of species to investigate how best to germinate the seeds and grow them on to plug plant size to enable them to be transplanted into the field (Kew, no date; BBC, 2011).

### 6.1.2 Aims and Objectives

Aims:

To identify the best laboratory techniques and media for axenic seed germination of *Dactylorhiza fuchsii*.

To identify the best laboratory techniques and media for growing plantlets to maturity (when they have true leaves and have reached an appropriate size for eventual transplantation to the field).

Objectives:

To compare germination rates on two types of media (symbiotic and asymbiotic), using axenic seed germination techniques.

To compare growth rates of protocorms on the same two types of media, using axenic methods.

(a)

(b)

Figure 6.1 (a): *Dactylorhiza fuchsii* as found in species-rich meadows (b) in closer view.


Figure 6.2: *Dactylorhiza fuchsii.*

## 6.2 Materials and Methods

### 6.2.1 Collection and storage of seeds

Seed capsules were collected, with permission from the landowner, from a natural population of *Dactylorhiza fuchsii* occurring at Wren's Nest National Nature Reserve in the West Midlands. The seed capsules were ripe (i.e. not green), but had not dehisced, when collected by hand after dry weather in August 2012. The seeds were air dried and subsequently stored in a sealed, sterile, plastic test tube at 5$^{o}$C.

### 6.2.2 Preparation of media

Two types of media were used. The first was Western, a proprietary brand supplied by Western Orchid Laboratories, Australia. This medium has been developed to counteract changes in pH during the growth and development of seedlings and has mainly been used for the propagation of tropical orchid species (Western Orchids Laboratory, no date; Millner *et al.*, 2008). This medium is supplied as a powder and was prepared as per the suppliers instructions. The second was an oats medium of 4 g/l of ground porridge oats and tissue culture agar, which is routinely used for axenic orchid seed germination (Seaton and Ramsay, 2005; Malmgren, 2011; Seaton *et al.*, 2011).

#### 6.2.2.1 Germination experiment

Both media were autoclaved and then replicates prepared in 90 mm diameter sterile plastic Petri dishes. Six replicate plates were prepared for each treatment. Where appropriate for the experimental design (Section 6.2.3), a small square (approximately 1 cm by 1 cm) of fungi (Culture B1, a *Ceratobasidium* sp. supplied by P. Seaton) was added to the centre of the Petri dish and the seeds were then sown onto the surface of the media in the dish, at the same time, using the method described below (Section 6.2.3.1).

#### 6.2.2.2 Re-plating experiment

Both Western and oats media were prepared with and without banana pulp (at 60 g/l), to act as a sugar source for the protocorms and then with and without fungi (Table 6.1). Banana is commonly used as a sugar source in orchid re-plating media (Seaton and Ramsay, 2005; Millner *et al.,* 2008; Malmgren, 2011).

### 6.2.3 Addition of plant material

#### 6.2.3.1 Seed sowing

Seeds were surface sterilized by agitation in a 10% (v/v) bleach (sodium hypochlorite) solution for 20 minutes. After this period, when the seeds were beginning to sink, they were rinsed thoroughly with sterile deionized water. The seeds were then suspended in sterile deionized water to allow for even distribution across the prepared Petri dishes. For each Petri dish, a 3 ml pipette was filled with this suspension and emptied onto the Petri dish.

For the germination experiment, four treatments were compared: oats media with fungi, oats media without fungi, Western media with fungi and Western media without fungi, with six replicate plates for each treatment.

### 6.2.3.2 Re-plating

For the re-plating experiment, protocorms were chosen from the most successful media from the germination stage of the experiment and re-plated on to six replicates of each of the eight treatments (Table 6.1). Protocorms of a similar size were chosen, as far as possible. A similar number of protocorms were placed on each plate (approximately nine).

Table 6.1: Treatments for the re-plating experiment

| **Media** | **With/without banana added to media** | **With/without fungus added to media** |
|---|---|---|
| Western | + banana | + fungus |
| | | - fungus |
| | - banana | + fungus |
| | | - fungus |
| Oats | + banana | + fungus |
| | | - fungus |
| | - banana | + fungus |
| | | - fungus |

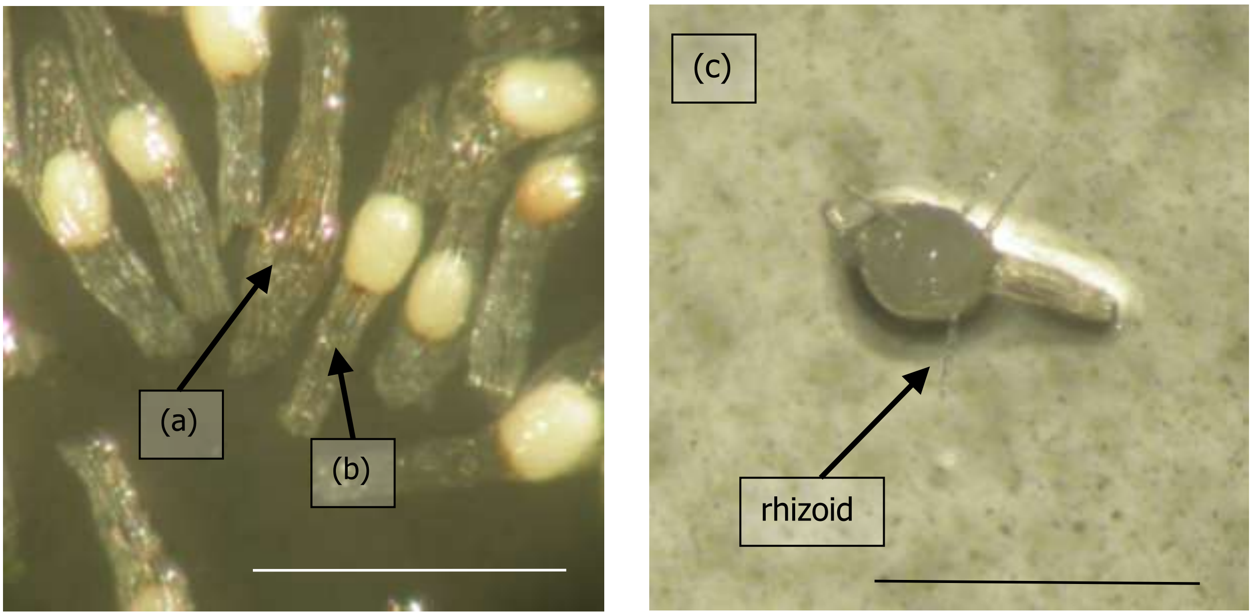
### 6.2.4 Observations and measurements

For the germination experiment, each plate was examined under a dissecting microscope and the developmental status of at least 150 of the seeds on each plate was recorded. The stages of development were recorded as: empty (no embryo), filled (embryo present, no germination) or germinated, i.e. now a protocorm (swollen embryo and testa split) after Millner *et al.* (2008; Figure 6.3). As *D. fuchsii* seeds do not display synchronous germination, observations were made on a weekly basis until it became clear that most seeds had germinated (eight weeks after the initial sowing).

For the re-plating experiment, photographs of all protocorms were taken using a Nikon E4500 SLR camera and MDC-A Relay Lens attached to a dissecting microscope, starting immediately after the protocorms were re-plated. Measurements of the lengths of the protocorms were calculated using Adobe Photoshop CS6 and Fiji 1.48[22] (Schindelin *et al.*, 2012). The increase in the growth of the individual protocorms after 15 weeks was calculated, this period being sufficient for a clear difference between treatments to be observed. An equal number of protocorms was randomly sampled from each plate. By the end of the first week, one set of plates (the Western+banana+fungi treatment) had an excessive growth of the added symbiotic fungi, which had grown thickly across the entire surface of each Petri dish and meant that measurements could not be taken for this

[22] Fiji is an image processing package from SciJava available as open source software.

treatment set. Consequently, this treatment was excluded from the statistical analysis, as its inclusion would mask other, more subtle, trends in the data.




(a) empty seed; (b) filled seed; (c) embryo has swollen and burst its *testa,* i.e. germinated (rhizoids also showing on this example). Scale bars represent 1 mm.

Figure 6.3: Photographs of unfilled, filled and germinated *Dactylorhiza fuchsii* seeds.

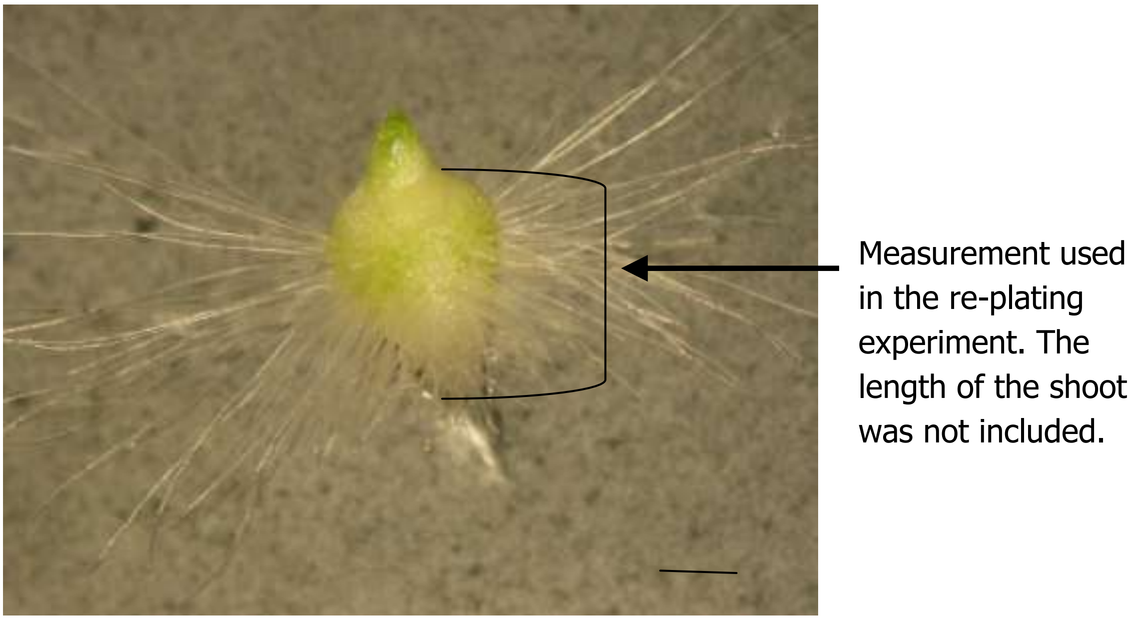



Figure 6.4: Photograph of *D. fuchsii* protocorm with developing shoot, showing measurement used in the re-plating experiment. Scale bars represent 1 mm.


Figure 6.5: Young *D. fuchsii* plantlets after transfer into honey jars on oats without banana (a) and Western without banana media (b).

For the germination experiment, the mean percentage germination per plate was calculated for each treatment. Percentage germination was calculated using the formula below (i.e. empty seed cases were not included).

$$\text{Percentage germination} = \frac{\text{number of germinated seeds}}{\text{number of filled seeds + number of germinated seeds}} \times 100$$

For the re-plating experiment, the mean increase in protocorm length (rather than width or any other dimension) was calculated for each treatment[23]. By week 15, some of the protocorms had shoots but others did not, therefore, for consistency across the protocorms, the shoot length was not included in the length measurement (Figure 6.4).

All statistical analysis was performed on SPSS Statistics for Windows Version 20.0 (IBM, 2011). One-way repeated measures ANOVA was used to compare the cumulative germination rates of the four media treatments over a five week period. The mean increases in protocorm length for the combined media treatments were compared at the end of the re-plating experiment with one-way ANOVA. The independent effects of the three separate media constituents (oats/Western; with/without banana; with/without fungi) and their interactions, were investigated with a three-way ANOVA.

[23] To avoid negative values in the data to be analysed, the most negative value was identified and the positive of this was added uniformly to the dataset, with the exception of known zeros.

Preliminary data exploration was carried out as outlined in the Methods (Section 2.5.3). Mauchly's sphericity test was used prior to the repeated measures ANOVA and the Huynh-Feldt version of this test was used for interpretation, as indicated by the sphericity test output.

The effect of plate was investigated as a data cleaning exercise prior to the one-way ANOVA on the re-plating experiment results as a routine check to see if there was any significant effect of plate. Plate was initially included as a random factor to discount its effect. However, with a very low p-value [$F(5,30) = 8.135$, $p < 0.001$)], its effect was stronger than that of treatment [$F(6,30) = 2.023$, $p = 0.094$], due to a substantial number of zeros in two sets of plates caused by plates drying up. Removal of these complete sets of plates from the analysis resolved the issue, with no consequent significant plate effect.

## 6.3. Results

### 6.3.1 Germination experiment

Germination rates for the different media with and without fungi are presented in Figure 6.6, from which it can be seen that 'oats with fungi' produced the highest percentage germination rate. Germination was also achieved more rapidly on this medium (i.e. at week 4 it was the only medium with any germination recorded).

A repeated measures ANOVA on the log10 transformed data showed that 'week' had a significant effect (Figure 6.6) [$F$(3.493, 69.854) = 55.904, p <0.001(Huynh-Feldt)] and also that there was a significant interaction between week and treatment i.e. the effect of week is dependent on the treatment (medium) [$F$(10.478, 69.854) = 4.984 p <0.001 (Huynh-Feldt)].

The means for weeks were: week 1, 0.00 (95% CI, 0.00 to 0.00); week 2, 0.01 (95% CI, 0.0 to 0.02); week 3 (95% CI, 0.04 to 0.54); week 4, 0.58 (95% CI, 0.17 to 2.04) and week 5, 2.83 (95% CI, 1.39 to 5.76).

*Post hoc* Tukey tests (p <0.05) showed that oats with fungi (mean 1.72, 95% CI, 0.66 to 4.46; homogeneous subset 'b') produced percentage germination rates that were significantly higher than those produced by any of the other treatments (Western with fungi: mean, 0.05, 95% CI, 0.02 to 0.12; Western without fungi: mean 0.02, 95% CI, 0.01 to 0.06; Oats without fungi: mean 0.02, 95% CI, 0.01 to 0.04; homogeneous subset 'a').

Protocorms from oats with fungi were therefore chosen for the re-plating experiment.

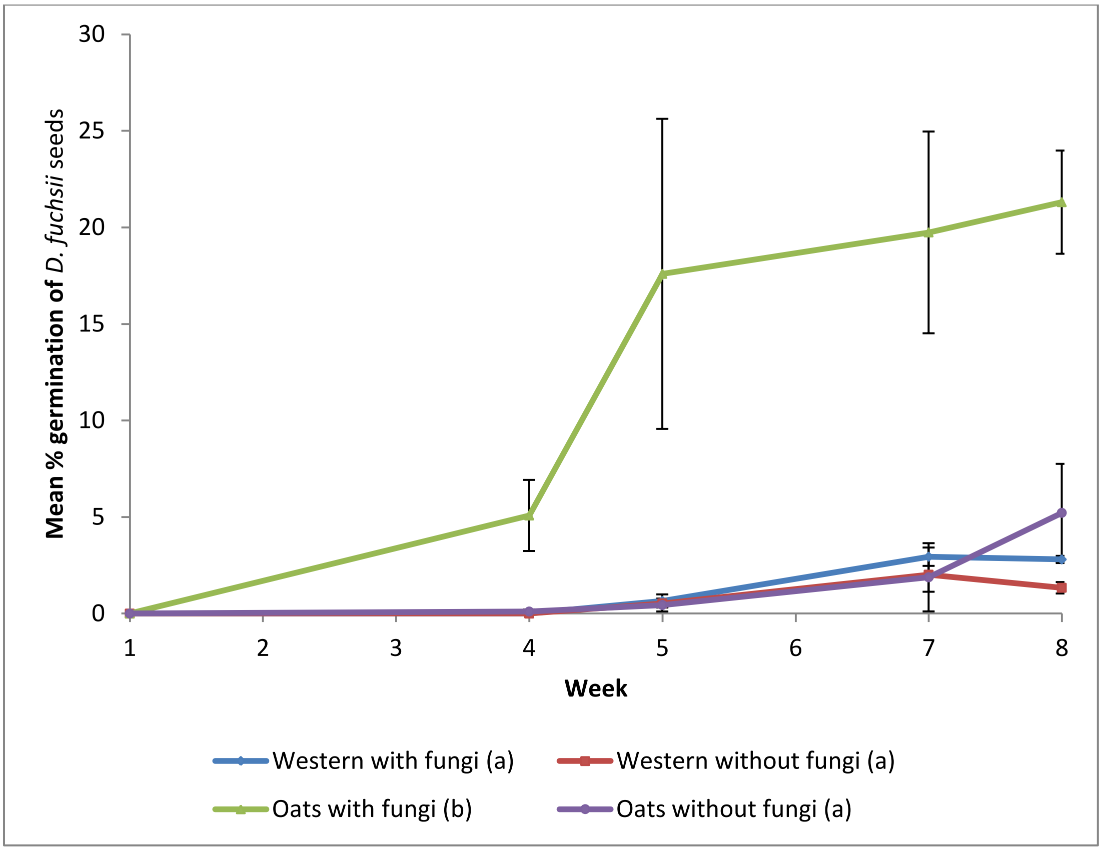



Figure 6.6: Results of media comparisons for seed germination of *D. fuchsii* after eight weeks on four media, six plates per treatment (mean percentage germination rates).

Notes:

Error bars represent ±1 S.E.

*Post hoc* Tukey tests on the transformed data following a repeated measures ANOVA [$F(7.841,52.271) = 4.984$ $p < 0.001$] showed that oats with fungi (homogeneous subset 'b') produced percentage germination rates that were significantly higher than those produced by any of the other treatments (homogeneous subset 'a'). Treatment means with the same label (a or b) are not significantly different from one another.

### 6.3.2 Re-plating experiment

A one-way ANOVA of the mean treatment data per plate showed no significant differences between the complete treatments. However, a further investigation was carried out into the independent effects of the three separate media constituents (oats/Western; with/without banana; with/without fungi) with the effect of plate discounted. A summary of the results is presented in Table 6.2.

Table 6.2: Investigation of the independent effects of the three treatment components (media: Western/Oats; with/without banana; with/without fungi) and their interactions

| **Source of variation** | **df** | **SS** | **MS** | ***F*** | **p** | |
|---|---|---|---|---|---|---|
| Media | 1 | 3.559 | 3.559 | 4.714 | 0.036 | * |
| Banana | 1 | 0.224 | 0.224 | 0.297 | 0.589 | |
| Fungi | 1 | 0.127 | 0.127 | 0.168 | 0.684 | |
| Plate (covariate) | 1 | 7.182 | 7.182 | 9.513 | 0.004 | ** |
| Residual (error) | 37 | 27.933 | 0.755 | | | |
| Total | 41 | 39.803 | | | | |

Notes:

* indicates where significance levels are $p < 0.05$.

** indicates where significance levels are $p < 0.01$.

The type of media [$F(1,37) = 4.714$, $p < 0.05$] had a significant effect on the growth of the protocorms, oats 1.88 (95% CI, 0.82 to 4.30) having a larger mean and therefore a larger increase in protocorms size than Western 0.46 (95% CI, 0.17 to 1.27). Neither the presence or absence of banana nor the presence or absence of fungi had a significant effect, although 'with banana' 1.11 (95% CI, 0.40 to 3.06) and 'with fungi' 1.06 (95% CI, 0.39 to 2.93) had larger mean increases in protocorm length than the 'without' treatments (0.78 (95% CI, 0.34 to 1.79); 0.82 (95% CI, 0.36 to 1.87) for banana and fungi, respectively).

## 6.4. Discussion

Meadow creation and restoration projects have often included orchids as target species and organisations such as Thompson and Morgan (Thompson and Morgan, 2014), the Hardy Orchid Society and Plantlife have grown species of British terrestrial orchids from seed and undertaken some reintroduction projects (HOS, 2014). However, little has been published on the germination and growth of orchids such as *D. fuchsii,* a species commonly found in meadows, and particularly not on more recently developed tissue culture media such as Western. This medium has previously been used mainly for the propagation of tropical orchid species (Western Orchids Laboratory, no date; Millner *et al.,* 2008). As such, the aim of the current study was therefore to compare Western medium with the commonly recommended oats medium to investigate its suitability for the propagation of *D. fuchsii.* Testing of new media is important to ensure the most effective method of orchid cultivation for the *ex situ* conservation of species from threatened habitats and also to provide a source of plants for reintroduction into newly created and restored sites.

The results demonstrate that oats produced higher axenic seed germination rates (mean at week eight: 21.30% and 5.22%; Figure 6.6) than Western media (mean at week eight: 2.80% and 1.34%), both with and without fungi. Also, that oats with the addition of fungi produced significantly higher germination rates than all the other media. The beneficial effect of the addition of symbiotic fungi on the oats media had been expected from previous studies (e.g. Knudson, 1922; Seaton and Ramsay, 2005; Malmgren, 2011), but the combination was included for comparison.

The results for the re-plating experiment demonstrated that oats medium was again more suitable than Western medium for growing on *D. fuchsii* protocorms, producing the greatest increase in growth of protocorms after 15 weeks of growth (1.88 mm; Table 6.2). The presence or absence of banana did not have a significant effect on the growth of the protocorms. This was more surprising, as the literature suggests that adding a source of complex carbohydrate, such as banana, has a beneficial effect on the growth of this (and many other British terrestrial orchid) species (Seaton and Ramsay, 2005; Malmgren, 2011; Seaton *et al.*, 2011). The presence/absence of fungi also did not have the expected significant effect. This may have been due to the protocorms having been sourced from the oats-with-fungi germination media and thus having fungi present already. It is also possible that the beneficial effect of the fungi is at the germination stage and not during protocorm growth. Orchid seeds do not have endosperm (nutrition) to supply the energy needed for germination, meaning that in the wild they depend entirely on symbiotic mycorrhizae for germination to occur (Rasmussen, 1995; Arditti and Ghani, 2000; Smith and Read, 2008). The role of these fungi in mature orchid plants is less understood (McCormick *et al.*, 2004) and there is some evidence that different fungi are associated with an orchid species at different stages of maturation (Dearnaley, 2007) or as environmental conditions change (McCormick *et al.*, 2006). The degree/existence of the specificity of fungal partners for photosynthetic orchids is contentious (Otero *et al.*, 2002): some studies have found that terrestrial photosynthetic orchids are associated with several different mycorrhizae (e.g. Zettler *et al.*, 2004), whereas others have found a much narrower range of species (e.g. Masuhara and Katsuya, 1994). The reason for these differences may be due to differences in cultural techniques and also due to the difficulty in identifying the cultured fungi (McCormick *et al.*, 2004). Confirmation of the fungal specificity of individual orchid species requires isolation and identification of the fungi (for which DNA sequencing may be required) and re-establishment of functioning mycorrhizal interactions (Dearnaley, 2007). The identification of fungi needed for orchid germination is important to aid *in vitro* cultivation, but also because fungal specificity may affect the distribution, population size and genetic diversity of orchids (McCormick *et al.*, 2004).

In summary, this study has demonstrated that, for the meadow orchid species *D. fuchsii*, oats medium produced a higher percentage germination rate than Western medium, both with and without fungi. Germination also occurred more rapidly on oats medium, particularly on oats with fungi. Oats medium with fungi produced significantly higher germination rates than all other treatments.  With regard to protocorm growth after re-plating, the experiment showed oats was again the more suitable medium. This suggests that for growing *D. fuchsii in vitro,* oats with fungi would be the best choice of the media tested for germination and oats would be the better choice than Western for the re-plating of protocorms. The presence or absence of banana and fungi were not significant, although 'with banana' and 'with fungi' had the larger mean increase in protocorm length compared to the 'without' treatments.

The successful growth of such protocorms, and their subsequent *ex vitro* transfer to appropriate meadow sites, could contribute to the conservation of the species, the biodiversity of the individual meadow site and also the restoration of this important and threatened habitat. This will be a topic for future studies, although transfer of plantlets out of honey jars to compost is proving to be difficult, as found in other studies (Ramsay and Stewart, 1998) and mentioned in the introduction to this chapter.

# Chapter 7

# General Discussion

## 7.1 Introduction

The focus of this study was to investigate methods of increasing the numbers of species in created MG5 meadows, particularly as some are known to perform poorly (Pywell *et al.,* 2003; Hewins *et al.,* 2012; Section 1.8). The methods tested were: increasing the number of species (and their frequency and abundance) at initial creation by undertaking multiple strewing of green hay; increasing the number of species (and their frequency and abundance) in established enhanced grasslands by carrying out green hay strewing; investigating the effect of disturbance and grazing in combination with green hay strewing and, lastly, comparing media types for laboratory culture of a meadow orchid species, such that new plants can ultimately be used to enhance created grasslands.

Six species (*Trisetum flavescens, Lathyrus pratensis, Leontodon hispidus, Dactylorhiza fuchsii, Carex flacca* and *Conopodium majus*) were missing from all the experimental receiver meadows and present in all the source meadows at the onset of this study. *Trisetum flavescens* and *Leontodon hispidus* transferred to all three receiver meadows; *Lathyrus pratensis* and *Dactylorhiza fuchsii* transferred to two out of three; *Carex flacca* transferred once and *Conopodium majus* never transferred within the duration of the study.

Several species transferred to Castle Vale (Chapter 3) and were present at the other receivers before treatment and in their sources. These were: *Centaurea nigra, Hypochaeris radicata, Rumex acetosa, Luzula campestris, Rhinanthus minor, Lotus corniculatus, Prunella vulgaris, Leucanthemum vulgare, Trifolium pratense* and *Anthoxanthum odoratum*. Several other species were present at Castle Vale before treatment, increased after treatment and were also present at all the other sites. These were: *Cerastium fontanum, Cynosurus cristatus*, *Festuca rubra*, *Holcus lanatus*, *Plantago lanceolata, Ranunculus acris, Achillea millefolium, Agrostis capillaris* and *Trifolium repens. Lolium perenne, Bromus hordeaceus* and *Taraxacum* spp.*,* were also in this category except that they were not present at Pikes Farm. Although *Lotus corniculatus* transferred to Castle Vale, it decreased at both other receivers, despite high percentage frequencies at the sources.

Ten species (*Briza media, Primula veris, Betonica officinalis, Linum catharticum, Equisetum arvense, Ajuga reptans, Anacamptis morio, Neottia ovata, Vicia cracca, Stellaria graminea* and *Hypericum perforatum*) were missing from two receiver meadows by the end of the study and present in their source meadows. *Stellaria graminea* was the only species, of these 10, that transferred in both cases. *Briza media, Primula veris, Betonica officinalis* and *Linum catharticum* all transferred in one of the two experiments. The remaining five species did not transfer.

Two further species were missing from two receiver meadows (Cae Gross (Chapter 4) and Golden Field (Chapter 5)) by the end of the study, yet present in their source meadows, but they were also present in the other source and receiver (Castle Vale). *Heracleum sphondylium* transferred at Golden Field but not at Cae Gross and *Dactylis glomerata* did not transfer at either Golden Field or Cae Gross. However, both species increased at Castle Vale and, as this grassland was glyphosated prior to strewing, their reappearance can be considered to represent transfer. Additionally, *Prunella vulgaris* was transferred at Castle Vale and Golden Field and the percentage frequency stayed approximately the same at Cae Gross (2% frequency recorded at Pikes Farm).

Several other species were present at one source and absent from its receiver but were present at another source and its receiver before treatment. *Ranunculus bulbosus* transferred to Golden Field and increased at Castle Vale. *Poa pratensis* and *Potentilla erecta* transferred to Golden Field and decreased at Cae Gross. *Euphrasia* sp. was present at both Golden Field and Cae Gross and their source meadows, but increased in frequency at Cae Gross and decreased at Golden Field.

There were also species at only one of each of the source meadows, which were not initially present at their receiver meadow. *Alopecurus pratensis, Avenula pubescens, Crepis biennis, Leontodon saxatile, Tragopogon pratensis* and *Arrhenatherum elatius* all transferred to Castle Vale, whereas

*Phleum pratense,*, *Allium vineale*, *Crataegus monogyna*, *Filipendula ulmaria*, *Galium palustre*, *Galium verum*, *Hordeum secalinum*, *Medicago lupis, Ophioglossum vulgatum, Ophrys apifera, Plantago media, Potentilla reptans, Schedonorus pratensis, Silaum silaus* and *Succisa pratensis* did not. *Poa trivialis* was present at Eades and at Castle Vale (Chapter 3) before treatment and increased after treatment.

*Platanthera chlorantha* and *Myosotis arvensis* were present at Pikes Farm and not at Cae Gross (Chapter 4) before treatment and were transferred. *Myosotis arvensis* also increased at Golden Field after treatment, even though it was not recorded at the Three Yew Trees*. Ranunculus repens* decreased at Cae Gross, despite being present at Pikes Farm (but at low frequency). However, *Carex* sp., *Crepis capillaris, Juncus articulatus, Juncus conglomeratus* and *Silene flos-cuculi* did not transfer to Cae Gross. *Polygala vulgaris* and *Ranunculus bulbosus* were present at Three Yew Trees and not at Golden Field before treatment and did not transfer. *Ranunculus bulbosus* was also present at Castle Vale before treatment and at its source meadow and increased in frequency after treatment.

## 7.2 Species transferability in green hay strewing

An examination of the species that transferred from source to receiver sites in this study (Table 7.1) suggests that percentage frequency at the source is a key factor in deciding the transferability of a species. This is indicated by the data in each chapter and in the amalgamated data. The species that did transfer to Castle Vale had a mean percentage frequency of 64.38% at the source meadow; to Cae Gross, 18.89% and to Golden Field, 32.5%. The species that did not transfer to Castle Vale had a mean percentage frequency of 21.31% at the source meadow; to Cae Gross, 10.00% and to Golden Field, 22.55%. That is, the species that did transfer had a higher overall percentage frequency at the source meadow than those that did not transfer, from the same source. However, the mean percentage frequency of the species that did transfer to Cae Gross from its source was less than that of the species that did not transfer to Castle Vale and Golden Field.

Table 7.1: A comparison of the results of species transfer in the three hay strewing experiments in this thesis

Ea – Eades meadow, CV – Castle Vale, Pi – Pikes Farm, CG – Cae Gross, 3YT – Three Yew Trees, GF – Golden Field; So- source meadow, Re – receiver meadow; T: transferred, DNT: Did not transfer, Incr: Increased, Decr: Decreased, Same: Stayed the same

| | **Percentage frequency in source meadow (So) and result of hay strewing in receiver meadow (Re)** | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | **So** | **Re** | **So** | **Re** | **So** | **Re** | **MG5 Constancy** | **Desir-ability** |
| **Species** | **Ea** | **CV** | **Pi** | **CG** | **3YT** | **GF** | | |
| *Cynosurus cristatus* | 84 | Incr | 18 | Incr | 8 | Same | V | D |
| *Festuca rubra* agg. | 84 | Incr | 48 | Incr | 88 | Decr | V | D |
| *Lotus corniculatus* | 100 | T | 96 | Decr | 84 | Decr | V | D |
| *Plantago lanceolata* | 100 | Incr | 80 | Incr | 96 | Incr | V | D |
| *Agrostis capillaris* | 96 | Incr | 100 | Same | 96 | Incr | IV | D |
| *Anthoxanthum odoratum* | 100 | T | 98 | Same | 100 | Same | IV | D |
| *Centaurea nigra* | 90 | T | 96 | Incr | 70 | Incr | IV | D |
| *Dactylis glomerata* | 44 | Incr | 8 | DNT | 40 | DNT | IV | N |
| *Holcus lanatus* | 92 | Incr | 88 | Incr | 46 | Same | IV | D |
| *Trifolium pratense* | 88 | T | 98 | Same | 96 | Incr | IV | D |
| *Trifolium repens* | 18 | Incr | 48 | Decr | 36 | Decr | IV | D |
| *Achillea millefolium* | 2 | Incr | 2 | Same | 32 | DNT | III | D |
| *Hypochaeris radicata* | 62 | T | 58 | Incr | 38 | Incr | III | D |
| *Lolium perenne* | 20 | Incr | 0 | Same | 2 | Same | III | D |
| *Luzula campestris* | 16 | T | 12 | Incr | 18 | Decr | III | D |
| *Prunella vulgaris* | 94 | T | 2 | Same | 10 | T | III | D |
| *Ranunculus acris* | 38 | Incr | 70 | Same | 84 | Incr | III | D |
| *Ranunculus bulbosus* | 18 | Incr | - | - | 8 | T | III | D |
| *Rumex acetosa* | 18 | T | 40 | Incr | 20 | Same | III | D |

(cont.)

| | Percentage frequency in source meadow (So) and result of hay strewing in receiver meadow (Re) | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | **So** | **Re** | **So** | **Re** | **So** | **Re** | **MG5 Constancy** | **Desir-ability** |
| **Species** | **Ea** | **CV** | **Pi** | **CG** | **3YT** | **GF** | | |
| *Scorzoneroides autumnalis* | 24 | Decr | 4 | Decr | 2 | Incr | III | D |
| *Taraxacum* spp. | 86 | Incr | 0 | Same | 38 | Same | III | D |
| *Trisetum flavescens* | 58 | T | 26 | T | 30 | T | III | D |
| *Arrhenatherum elatius* | 64 | T | - | - | - | - | II | N |
| *Briza media* | 90 | T | | - | 20 | DNT | II | D |
| *Cerastium fontanum* | 14 | Incr | 56 | Incr | 30 | Incr | II | D |
| *Cirsium arvense* | - | - | 0 | Decr | - | - | II | U |
| *Galium verum* | 86 | DNT | - | - | - | - | II | D |
| *Heracleum sphondylium* | 72 | Incr | w/o | DNT | 18 | T | II | D |
| *Lathyrus pratensis* | 14 | T | 10 | T | 8 | DNT | II | D |
| *Leontodon hispidus* | 88 | T | 72 | T | 100 | T | II | D |
| *Leucanthemum vulgare* | 86 | T | w/o | Same | 2 | DNT | II | D |
| *Poa pratensis* | - | - | 14 | Decr | 34 | T | II | D |
| *Poa trivialis* | 2 | Incr | - | - | - | - | II | D |
| *Primula veris* | 86 | T | - | - | w/o | DNT | II | D |
| *Rhinanthus minor* | 88 | T | 98 | Incr | 98 | Decr | II | D |
| *Trifolium dubium* | - | - | 26 | Incr | 0 | Same | II | D |
| *Agrostis stolonifera* | - | - | 0 | Decr | - | - | I | U |
| *Alopecurus pratensis* | 2 | T | - | - | - | - | I | N |
| *Bellis perennis* | 2 | DNT | 0 | Decr | 0 | Incr | I | D |
| *Betonica officinalis* | 36 | DNT | 8 | T | - | - | I | D |
| *Bromus hordeaceus* | 6 | Incr | 0 | Same | 6 | Incr | I | N |
| *Carex flacca* | 88 | DNT | 6 | T | 2 | DNT | I | D |
| *Conopodium majus* | 12 | DNT | 46 | DNT | 58 | DNT | I | D |
| *Crepis capillaris* | - | - | 4 | DNT | - | - | I | D |
| *Filipendula ulmaria* | 34 | DNT | - | - | - | - | I | N |
| *Juncus articulatus* | - | - | 12 | DNT | - | - | I | N |
| *Juncus effusus* | - | - | 0 | Same | - | - | I | N |
| *Ophioglossum vulgatum* | 4 | DNT | - | - | - | - | I | D |
| *Phleum pratense* | 6 | DNT | 0 | - | 0 | Decr | I | D |
| *Plantago media* | 2 | DNT | - | - | - | - | I | D |
| *Potentilla erecta* | - | - | 40 | Decr | 4 | DNT | I | D |
| *Potentilla reptans* | 2 | DNT | - | - | - | - | I | D |
| *Ranunculus repens* | - | - | 4 | Decr | 0 | Same | I | U |
| *Schedonorus pratensis* | 52 | DNT | - | - | - | - | I | D |
| *Silaum silaus* | 10 | DNT | - | - | - | - | I | D |
| *Succisa pratensis* | 10 | DNT | - | - | - | - | I | D |
| *Vicia cracca* | 32 | DNT | - | - | 42 | DNT | I | N |
| *Ajuga reptans* | 14 | DNT | - | - | 2 | DNT | - | D |
| *Allium vineale* | 18 | DNT | - | - | - | - | - | N |
| *Anacamptis morio* | 24 | DNT | - | - | 38 | DNT | - | D |
| *Avenula pubescens* | 66 | T | - | - | - | - | - | N |

(cont.)

| | Percentage frequency in source meadow (So) and result of hay strewing in receiver meadow (Re) | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | So | Re | So | Re | So | Re | MG5 Constancy | Desir-ability |
| Species | Ea | CV | Pi | CG | 3YT | GF | | |
| *Carex* sp. | - | - | 2 | DNT | - | - | - | - |
| *Crataegus monogyna* | 2 | DNT | - | - | - | - | - | U |
| *Crepis biennis* | 80 | T | - | - | - | - | - | N |
| *Dactylorhiza fuchsii* | 46 | DNT | 16 | T | 56 | T | - | D |
| *Equisetum arvense* | 2 | DNT | 4 | DNT | - | - | - | U |
| *Euphrasia* sp. | - | - | 96 | Incr | 28 | Decr | - | D |
| *Galium palustre* | 2 | DNT | - | - | - | - | - | D |
| *Hordeum secalinum* | 2 | DNT | - | - | - | - | - | N |
| *Hypericum perforatum* | - | - | 2 | DNT | w/o | DNT | - | N |
| *Juncus conglomeratus* | - | - | 8 | DNT | - | - | - | N |
| *Leontodon saxatile* | 14 | T | - | - | - | - | - | D |
| *Linum catharticum* | 16 | DNT | w/o | T | - | - | - | D |
| *Medicago lupis* | 44 | DNT | - | - | - | - | - | D |
| *Myosotis arvensis* | - | - | 2 | T | 0 | Incr | - | D |
| *Nardus stricta* | - | - | 0 | Same | - | - | - | N |
| *Neottia ovata* | 2 | DNT | - | - | w/o | DNT | - | D |
| *Ophrys apifera* | 6 | DNT | - | - | - | - | - | D |
| *Platanthera chlorantha* | - | - | 24 | T | - | - | - | D |
| *Polygala vulgaris* | - | - | - | - | w/o | DNT | - | D |
| *Quercus robur* seedling | - | - | 0 | Same | 2 | Decr | - | U |
| *Rumex obtusifolius* | - | - | 4 | DNT | 0 | Incr | - | U |
| *Silene flos-cuculi* | - | - | w/o | DNT | - | - | - | D |
| *Stellaria graminea* | - | - | 6 | T | 4 | T | - | N |
| *Tragopogon pratensis* | 48 | T | - | - | - | - | - | N |

The species of interest are those that do not follow this pattern, i.e. species with a high percentage frequency at the source but did not transfer or those that had a low percentage frequency at the source, but did transfer. Of the species that transferred at low percentage frequencies (Table 7.2), *L. campestris, P. vulgaris, R. bulbosus* and *P. trivialis* are relatively common, generalist species that can be found in a wide range of grasslands. Therefore, it is not surprising that they established, even at low percentage frequencies in the source, as they could have colonized from grassland surrounding the receiver meadows. *Lathyrus pratensis* is a relatively common species in meadows, but it did not appear to persist in either of the two receivers to which it transferred (possibly due to chance events, exacerbated by its low frequency, to unsuitable site conditions or, to not being sampled if it was still present but at low frequency or other unknown reason). *Alopecurus pratensis* and *Leontodon saxatile* are perhaps more surprising as they are less generalist species. The former likes damp conditions (an explanation for this species is suggested in Section 3.4.2) and the latter likes dry, sandy or calcareous conditions (Rose, 1981). However, neither species persisted in the receiver (Castle Vale; Chapter 3).

Table 7.2: Species that transferred at low percentage frequencies
Associated information is from Grime *et al.* (1988)

| **Species** | **Associated floristic diversity** | **Established strategy** | **Flowering time and duration (months)** | **Seed weight** | **Other information** |
|---|---|---|---|---|---|
| *Alopecurus pratensis** | Medium | C/CSR | April, 3 | Medium | - |
| *Lathyrus pratensis*† | Relatively high | CSR | May, 4 | Heavy | Requires scarification |
| *Leontodon saxatile** | Relatively low | S/SR | June, 4 | Relatively light | - |
| *Luzula campestris*† | Medium | S/CSR | March, 4 | Medium | - |
| *Prunella vulgaris* | High | CSR | June, 4 | Medium | - |
| *Ranunculus bulbosus* | High | SR/CSR | May, 2 | High | Requires chilling |
| *Poa trivialis* | Relatively low | CR/CSR | June, 1 | Low | - |

*Species only present at one source (i.e. 1 successful establishment from 1 attempt).
†MG5 species identified as performing poorly from a review of the literature, (Chapter 1, Table 1.1).

Additionally, *Platanthera chlorantha* transferred despite a relatively low percentage frequency at the source meadow. *P. chlorantha* is an orchid, so it is therefore surprising that this species established so quickly from transfer, as mentioned in the discussions of the meadow chapters (3-5; Trueman and Millett, 2003; Kotilinek *et al.,* 2015). However, only one plant was observed and it was recorded on the walkover, rather than in a quadrat (and therefore in the smaller, sampled, portion of the meadow). *Bromus hordeaceus* increased (i.e. was either transferred or spread from existing plants) at two receivers, despite low percentage frequencies at the sources. This is probably not surprising, as it is a ruderal species that is common in meadows (Grime *et al.,* 1988). It flowers from May for three months, meaning that seed would be available at the time of the hay cut, but it does have a heavy seed (Grime *et al.,* 1988), which could be lost from the hay.

*Euphrasia* sp. increased in percentage frequency (i.e. transferred) at one receiver, where the percentage frequency at the source was very high and decreased (i.e. did not transfer) at another, despite a fairly high percentage frequency at the source. *Euphrasia* is a genus of annual, hemiparasitic species (Stace, 2011), which has a relatively high associated floristic diversity and pasture is its most common habitat (Grime *et al.,* 1988). It is an SR strategist, flowers from June and has a low seed weight, which needs chilling to aid germination (Grime *et al.,* 1988). It is therefore surprising that it decreased at Golden Field, although one possible reason is the disturbance treatment at this site, as the species is associated with sites where there is no disturbance (Grime *et al.,* 1988).

Six species were inconsistent in their pattern of transfer (Table 7.3), sometimes transferring at low source percentage frequencies and sometimes not transferring at high source percentage frequencies (Table 7.1). This could be due to several reasons including: a narrow range of suitable conditions that were present in some receivers but not others, seed being lost in transit, either because it is very light or very heavy, and seed not being available at the time of the hay cut in that meadow in that year (e.g. due to the timing of the cut or that it had been eaten etc.). Possibly not enough time has elapsed from creation for the species to become established, or the species was present but was not recorded.

Table 7.3: Species that transferred inconsistently
Associated information is from Grime *et al.* (1988)

| **Species** | **Associated floristic diversity** | **Established strategy** | **Flowering time and duration (months)** | **Seed weight** | **Other information** |
|---|---|---|---|---|---|
| *Achillea millefolium* | Relatively high | CR/CSR | June, 3 | Light | - |
| *Betonica officinalis*† | High | S | June, 4 | Relatively heavy | - |
| *Dactylorhiza fuchsii* | High | S/CSR | June, 2 | Dust-like | Benefits from mycorrhizae |
| *Linum catharticum* | Relatively high | SR | June, 4 | Light | Needs chilling |
| *Scorzoneroides autumnalis* | Medium | R/CSR | May, 3 | Medium | Needs dry conditions |
| *Trifolium repens* | Medium | CR/CSR | June, 4 | Relatively high | Needs scarification |

†MG5 species identified as performing poorly from a review of the literature, (Chapter 1, Table 1.1).

*Bellis perennis* did not transfer to one receiver, where the source percentage frequency was low and increased at another receiver, despite no records at source. *B. perennis* is a common grassland species that is an R/CSR strategist (Grime *et al.,* 1988), therefore it is surprising that it has not colonized all the receivers from surrounding grassland, even if not from the source meadows. It also decreased in the remaining receiver meadow (not being present in that receiver's source). However, it is only a constancy I (0-20% frequency) species in MG5 grasslands (Rodwell, 1992), so perhaps there is some element of the management of these meadows that does not suit this species. It is also a low-growing species, which may have been shaded out by the tall vegetation on some of the receiver meadows.

*Lotus corniculatus* had a low transfer rate, only transferring once in three attempts (one transfer and two 'decrease in amounts'), despite high percentage frequencies at the source meadows. As mentioned previously, this species has been found to be difficult to establish in previous studies (Hopkins *et al.,* 1999; Smith *et al.,* 2000; Besenyei, 2000; Hofmann and Isslestein, 2004; Rayner, 2005; Sections 3.4.2, 4.4.4, 5.4.4) possibly due to late seed set date, heavy seed that is lost from the hay *en route* and that it can be a low growing species, with just a moderate number of large seeds produced only infrequently (Grime *et al.,* 1988).

Seven species did not transfer, despite relatively high percentage frequencies in the source (Table 7.4). As mentioned above and in the relevant chapters (3-5), orchids can take time to appear in created meadows, which may be why *A. morio* has yet to be recorded. Four of the species that did not transfer were only present in one source meadow, making it more likely that this could be a chance event for these species. However, *F. ulmaria* has a preference for wet conditions, which may be why this species was not successfully transferred, as the receivers were relatively dry. Of the remaining two species, *Vicia cracca* has heavy seeds, which may have been lost in transit. *Conopodium majus* is an early flowering species, with heavy seeds, which are therefore likely to have been lost from the stalk by the time of the hay cut. As mentioned in Section 4.5.3, other studies have also been unsuccessful in establishing this species, although Trueman and Millett (2003) and Smith *et al.* (2000) were successful. It is noteworthy that five out of the seven species flower relatively early (in May; Grime *et al.,* 1988). Of the remaining species, one has light seed (and flowers relatively late), another has heavy seed and the last species (*F. ulmaria*) has a requirement for specific environmental conditions.

*Dactylis glomerata* did not transfer from two source meadows, despite a relatively high percentage frequency in one of the sources. It was already present in the remaining receiver (Castle Vale) and increased in frequency at this site. It is surprising that this species did not perform more successfully, as it is a competitive species that flowers in May, meaning that seed would have been available at the time of the hay cut, and other studies have been successful (Trueman and Millett, 2003).

Table 7.4: Species that did not transfer, despite relatively high percentage frequencies at two sources
Associated information is from Grime *et al.* (1988)

| **Species** | **Associated floristic diversity** | **Established strategy** | **Flowering time and duration (months)** | **Seed weight** | **Other information** |
|---|---|---|---|---|---|
| *Anacamptis morio* | High | S/SR | May, 2 | Dust-like | Benefits from mycorrhizae |
| *Conopodium majus*† | Medium | S/CSR | May, 2 | Heavy | Needs chilling |
| *Filipendula ulmaria** † | Relatively low | C/SC | June, 3 | Medium | Likes wet conditions |
| *Galium verum** † | High | SC/CSR | July, 2 | Relatively light | - |
| *Medicago lupis** | Relatively high | R/SR | May, 4 | Heavy | Needs scarification |
| *Schedonorus pratensis** | Medium | CSR | May, 3 | Relatively light | - |
| *Vicia cracca*† | Relatively low | C/CSR | June, 3 | Very heavy | Needs scarification |

*Species only present at one source (i.e. 1 success/1 attempt).
†MG5 species identified as performing poorly from a review of the literature, (Chapter 1, Table 1.1).

*Primula veris* is noteworthy as it transfers well (in this and other studies e.g. (Besenyei, 2000; Trueman and Millett, 2003; Rayner, 2005), even though it flowers early. This is thought to be because the seeds are held in the seed head on a robust stalk, which holds the seed until after the hay cut (Besenyei, 2000). *Centaurea nigra* is also notable, as, although it sets seed late (Grime *et al.,* 1988), it does not normally perform poorly.

The remaining noteworthy species is *Potentilla erecta*, which decreased at one site despite high percentage frequency at the source (and also did not transfer from one source with low percentage frequency). A possible reason for the lack of transfer, is that lime was applied to the receiver meadow (Golden Field), which, may have made conditions less suitable for this particular species, which is adapted to soil with a low pH (Grime *et al.,* 1988). It is also a low-growing species, meaning its seeds may have been missed by the baling equipment.

There are 16 species described above as performing poorly or inconsistently in this study, seven of which were on the list of MG5 species which perform poorly from a review of the literature (Chapter 1, Table 1.1) and two of which were orchid species. Of these 16 species, six flower relatively early and one flowers relatively late, which is a possible reason for their lack of transfer.  This has been observed in other studies (Besenyei, 2000; Rayner, 2005; Edwards *et al.,* 2007). Strewing hay cut at different times of the year may aid in the transfer of these species, as has been noted by others (Jones *et al.,* 1995; Walker *et al.,* 2004; Rayner, 2005; Edwards *et al.,* 2007; Natural England, 2010a). However, this proved to be difficult during this study due to site owners concerns about the impact on the meadows and also difficulties due to contracts and/or expectations of graziers regarding their date of access to the meadows.

There are six MG5 constancy I species, one constancy II species, two constancy III species, two constancy IV species, one constancy V species and four species that are not on the MG5 species list, two of which are orchids, that performed poorly or inconsistently. That is, most of these species are only found at low frequencies in MG5 meadows. Most of these species are also stress-tolerators or stress-tolerance is part of their strategy. These are similar results to that of Pywell *et al.* (2003). It would therefore seem that although strewing hay on more than one occasion, either one year after the initial creation or into an established species-rich sward, has increased the number of species that have transferred, there is still a group of species that do not readily transfer. This could be because the conditions in created meadows are not yet similar enough to those in ancient species-rich meadows for these species. This could be due to the high fertility of restoration sites (Pywell *et al.,* 2003; McCrea *et al.,* 2004), the soil communities on these sites (Smith *et al.,* 2008; Dunn and Tallowin, 2012) or some other aspect of their functioning e.g. a lack of facilitator species. Species that are not stress-tolerators appear to have other specific reasons for their lack of transfer; e.g. *F. ulmaria* prefers specific environmental conditions and *L. corniculatus* and *V. cracca* have heavy seed that may have been lost from the hay before reaching the receiver site.

The loss of orchid habitat and other threats to these species mean that the development of methods to conserve them is important, including *ex situ* techniques (Swarts and Dixon, 2009; Kew, 2013) and the establishment of new populations derived from propagated orchids (McKendrick 1995; Scade *et al*. 2006; Ashmore *et al*., 2011; Krupnick *et al*., 2013). Although at least some of these species will transfer through green hay strewing, they can take several years to establish. It may also not be possible to collect green hay containing rarer species. The successful growth of orchids *in vitro*, and their subsequent *ex vitro* transfer to appropriate meadow sites, could contribute to the conservation of these species.

### 7.3 Study limitations

This thesis represents a very practical and practitioner-orientated Ph.D. project, done in collaboration with existing hay-strewing projects and researching methods in real-world situations. This meant, unfortunately, that full site control was not always achievable; if it had been achievable, the design of experiments, particularly at Castle Vale would have been more rigorous in terms of plot replication.  A further enhancement of the methodology, had it been possible, would have been to lay out the treatment blocks and then carry out the baseline survey, however, this was not possible due to the lack of full site and implementation (of the strewing) control. Longer-term monitoring of the experiments would reduce the impact of year to year variations in the vegetation, caused by uncontrollable factors such as the weather. However, as shown by the Cae Gross experiment, treatment blocks can merge together in the longer term, due to normal management operations of cutting, tedding and baling; therefore, treatment blocks may need to be separated by much larger buffer strips for long term monitoring of meadow experiments set in real-world situations.

Vegetation survey methodologies are inconsistent in the literature; for instance, a variety of quadrat sizes and numbers are used. For example, Stevenson *et al.* (1997) used the NVC survey method of 2 m by 2 m quadrats, Marriott *et al.* (2002) used point quadrats, Pywell *et al.* (2002) used 40 cm by 40 cm quadrats, Gilbert *et al.* (2003) used 1 $m^2$ quadrats and Holzel and Otte (2003) used 10 m by 10 m quadrats. Quadrat size, number and methodology are important in determining sensitivity to detecting change within the vegetation (Critchley and Poulton, 1998). Barnett and Stohlgren (2003) recommend "*a combination of large and small multi-scale (nested) and single scale plots for the most accurate recording of species richness*" (modified Whittaker design). Given more time for the monitoring of the receiver meadows, more quadrats could have been surveyed, at a variety of scales.

A number of other experiments were created and monitored during this thesis, some of which were replicates of experiments described here. Full analysis of these other experiments has not yet been carried out, but it should be noted that, when the analysis of the data from all the sites (including the ones described here) was at its initial stages, then the results of the same or similar experiments on different sites were not necessarily consistent. Word count limitations meant that these experiments could not be included here and, as mentioned, full analysis has not yet been carried out, but this should be borne in mind. If there are differences between the sites, potential reasons could include differences between the receiver sites (e.g. conditions being more suitable for some species at one site than at the other(s) or the potentially different ecotypes in the different source meadows making them more or less fit for the conditions in the receiver.

## 7.4 Conclusions

Green hay strewing created a vegetation resembling that of MG5 grassland communities after only 2-3 years where the existing vegetation had been killed by herbicide (Chapter 3). Strewing hay twice in consecutive years resulted in a vegetation with more species and species with higher frequencies compared with haying once.

Green hay strewing increased the number of species in an existing species-rich sward and also increased the frequency and abundance of existing species, at both Cae Gross and Golden Field. At Golden Field, the experiment showed that the relationships are complex. Hayed/not-grazed/high-disturbance had the highest mean percentage cover of desirable species and not-hayed/grazed/no-disturbance had the lowest. A larger experiment with greater statistical power might be able to detect more significant main effects.

Overall, species that transferred tended to have a higher percentage frequency in the source meadow than species that did not, but this was not the case for every species (i.e. some species with a high source percentage frequency did not transfer and, conversely, some species with a low source percentage frequency did transfer). Flowering/seed set date relative to the time of the hay cut appeared to be important with regards to the success of transfer. Species that performed well were generalist species and those that performed poorly were more specialist species.

Species were still missing from created swards (not transferred) by the end of the study. These tended to be those that are found at low frequencies in MG5 meadows and have stress-tolerance as part of their life strategy. Species that were not stress-tolerators tended to have specific possible reasons for their lack of success e.g. requirements for specific environmental conditions, seed weight or other factor(s) yet to be determined.

Longer-term monitoring may be needed to record the ultimate appearance of some species. The use of traditional management techniques enhanced the benefit of adding green hay to plots in a wider field, as the seeds were distributed more evenly over time.

For the laboratory based orchid germination and propagation experiment, oats medium produced higher axenic seed germination rates than Western medium, both with and without fungi. The addition of fungi produced significantly higher germination rates than all the other media. For protocorm growth after re-plating, media type had a significant effect on their growth with the protocorms on oats showing the greater increase in size than those on Western. Neither the presence or absence of banana nor the presence or absence of fungi had a significant effect, although 'with banana' and 'with fungi' had the larger mean increase in protocorm length compared to the 'without' treatments.

## 7.5 Possibilities for further work

There are several possibilities for further work related to this thesis. Continued monitoring of these sites would be beneficial to study if additional species appear after this study and also to monitor the spread (or otherwise) of the established species as well as changes to dominance and evenness. The experiments in this thesis should be repeated, if possible, with greater replications to investigate if the results are repeatable. Further strews of green hay could take place to develop existing created vegetation(s) and to investigate both the transfer of further additional species and the optimum time for further strewing from creation (and/or stage of development of the receiver meadow).

Similar experiments could also be run with either early- or late-cut hay, or with seed collected by hand at appropriate times, with the aim of increasing the transfer chances of poorly performing species that have early or late seed set times.

Investigation of the best method to transfer *in vitro* orchid plantlets out of honey jars and into compost is the next essential stage for introducing them into suitable grassland sites. This would require experiments on their transfer into compost, as well as introduction experiments with subsequent monitoring.

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## Appendix 2.1

## Full species list for all study sites, with key to abbreviations used in tables and species ordination diagrams

Nomenclature follows Stace (2011)

| | | | |
|---|---|---|---|
| *Achillea millefolium* | Ach mil | *Crepis capillaris* | Cre cap |
| *Agrostis capillaris* | Agr cap | *Crepis vesicara* | Cre ves |
| *Agrostis canina* | Agr can | *Cynosurus cristatus* | Cyn cri |
| *Agrostis stolonifera* | Agr sto | *Dactylis glomerata* | Dac glo |
| *Ajuga reptans* | Aju rep | *Dactylorhiza fuchsii* | Dac fuc |
| *Allium vineale* | All vin | *Deschampsia cespitosa* | Des ces |
| *Alopecurus geniculatus* | Alo gen | *Elytrigia repens* | Ely rep |
| *Alopecurus pratensis* | Alo pra | *Epilobium ciliatum* | Epi cil |
| *Anacamptis morio* | Ana mor | *Epilobium hirsutum* | Epi hir |
| *Anisantha sterilis* | Ani ste | *Equisetum arvense* | Equ arv |
| *Anthoxanthum odoratum* | Ant odo | *Euphrasia* sp. | Eup sp. |
| *Anthriscus sylvestris* | Ant syl | *Festuca rubra* agg. | Fes rub |
| *Aphanes arvensis* | Aph arv | *Filago vulgaris* | Fil vul |
| *Arrhenatherum elatius* | Arr ela | *Filipendula ulmaria* | Fil ulm |
| *Avenula pubescens* | Ave pub | *Fragaria* sp. | Fra sp. |
| *Bellis perennis* | Bel per | *Fraxinus excelsior* | Fra exc |
| *Betonica officinalis* | Bet off | *Galium aparine* | Gal apa |
| *Betula* sp. | Bet sp. | *Galium palustre* | Gal pal |
| *Brassicaceae* sp. | Bra sp. | *Galium verum* | Gal ver |
| *Briza media* | Bri med | *Genista tinctoria* | Gen tin |
| *Bromus hordeaceus* | Bro hor | *Geranium dissectum* | Ger dis |
| *Cardamine hirsuta* | Car hir | *Geranium molle* | Ger mol |
| *Cardamine pratensis* | Car pra | *Geum urbanum* | Geu urb |
| *Carex echinata* | Car ech | *Heracleum sphondylium* | Her sph |
| *Carex flacca* | Car fla | *Holcus lanatus* | Hol lan |
| *Carex leporina* | Car lep | *Holcus mollis* | Hol mol |
| *Carex nigra* | Car nig | *Hordeum secalinum* | Hor sec |
| *Carex panicea* | Car pan | *Hypericum perforatum* | Hyp per |
| *Carex* sp. | Car sp. | *Hypochaeris radicata* | Hyp rad |
| *Centaurea nigra* | Cen nig | *Iris pseudacorus* | Iri pse |
| *Cerastium fontanum* | Cer fon | *Juncus articulatus/acutiflorus* | Jun art |
| *Chamerion angustifolium* | Cha ang | *Juncus bufonius* | Jun buf |
| *Cirsium arvense* | Cir arv | *Juncus conglomeratus/effusus* | Jun con |
| *Cirsium palustre* | Cir pal | *Juncus inflexus* | Jun inf |
| *Cirsium vulgare* | Cir vul | *Lactuca serriola* | Lac ser |
| *Conopodium majus* | Con maj | *Lathyrus pratensis* | Lat pra |
| *Conyza* sp. | Con sp. | *Leontodon hispidus* | Leo his |
| *Corylus avellana* | Cor ave | *Leontodon saxatile* | Leo sax |
| *Crataegus monogyna* | Cra mon | *Leucanthemum vulgare* | Leu vul |
| *Crepis biennis* | Cre bie | *Linum catharticum* | Lin cat |

| | | | |
|---|---|---|---|
| *Lolium perenne* | Lol per | *Rubus fruticosus* agg. | Rub fru |
| *Lotus corniculatus* | Lot cor | *Rumex acetosa* | Rum ace |
| *Luzula campestris* | Luz cam | *Rumex acetosella* | Rum ace |
| *Medicago lupulina* | Med lup | *Rumex crispus* | Rum cri |
| *Myosotis arvensis* | Myo arv | *Rumex obtusifolius* | Rum obt |
| *Nardus stricta* | Nar str | *Rumex sanguineus* | Rum san |
| *Neottia ovata* | Neo ova | *Schedonorus pratensis* | Sch pra |
| *Odontites vernus* | Odo ver | *Scorzoneroides autumnalis* | Sco aut |
| *Ophioglossum vulgatum* | Oph vul | *Senecio jacobaea* | Sen jac |
| *Ophrys apifera* | Oph api | *Senecio vulgaris* | Sen vul |
| *Phleum pratense* | Phl pra | *Silaum silaus* | Sil sil |
| *Pimpinella saxifraga* | Pim sax | *Silene flos-cuculi* | Sil flo |
| *Plantago lanceolata* | Pla lan | *Sisymbrium officinale* | Sis off |
| *Plantago major* | Pla maj | *Sonchus asper* | Son asp |
| *Plantago media* | Pla med | *Spergula arvensis* | Spe arv |
| *Platanthera chlorantha* | Pla chl | *Stellaria graminea* | Ste gra |
| *Poa annua* | Poa ann | *Succisa pratensis* | Suc pra |
| *Poa pratensis* | Poa pra | *Taraxacum* spp. | Tar spp. |
| *Poa trivialis* | Poa tri | *Tragopogon pratensis* | Tra pra |
| *Polygala vulgaris* | Pol vul | *Trifolium dubium* | Tri dub |
| *Potentilla erecta* | Pot ere | *Trifolium medium* | Tri med |
| *Potentilla reptans* | Pot rep | *Trifolium pratense* | Tri pra |
| *Primula veris* | Pri ver | *Trifolium repens* | Tri rep |
| *Prunella vulgaris* | Pru vul | *Trisetum flavescens* | Tri fla |
| *Prunus spinosa* | Pru spi | *Urtica dioica* | Urt dio |
| *Pulicaria dysenterica* | Pul dys | *Veronica chamaedrys* | Ver cha |
| *Quercus* sp. | Que sp. | *Vicia cracca* | Vic cra |
| *Ranunculus acris* | Ran acr | *Vicia hirsuta* | Vic hir |
| *Ranunculus bulbosus* | Ran bul | *Vicia sativa* | Vic sat |
| *Ranunculus flammula* | Ran fla | *Vicia sepium* | Vic sep |
| *Ranunculus repens* | Ran rep | *Viola* sp. | Vio sp. |
| *Rhinanthus minor* | Rhi min | | |

# Appendix 2.2

## Photographs of machinery used

Figure 2.2.1: Grass/power harrow (Photo: Tierney, 2011; Golden Field and Cae Gross).

Figure 2.2.2: Tedder (Golden Field and Cae Gross) (Farm King, 2016).

(a)

(b)

(c)

Figure 2.2.3: (a) Bank Commander and baler, used on the Herefordshire sites (b) and (c) the bank commander and baler in use, with the bales that it produces. Bales are 52 cm (20") x 55 cm (22") in size (Tracmaster, 2014).

# Appendix 4.1
# Cae Gross and Pikes – comparison of species lists

Table 4.1.1: Range and mean of the abundances of the species found in Cae Gross and Pikes Farm SSSI

| Species | Abundance | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Max | Min | Mean | Max | Min | Mean | Max | Min | Mean | Max | Min | Mean | Max | Min | Mean | Max | Min | Mean |
| | Pi2011 | | | CG2011 | | | CG2012 | | | CG2013 | | | CG2014 | | | CG2015 | | |
| *Achillea millefolium* | 1.00 | 0.00 | 0.02 | 30.00 | 0.00 | 1.54 | 7.00 | 0.00 | 0.61 | 5.00 | 0.00 | 0.46 | 7.00 | 0.00 | 0.61 | 3.00 | 0.00 | 0.43 |
| *Agrostis capillaris* | 30.00 | 1.00 | 9.14 | 20.00 | 1.00 | 6.00 | 60.00 | 1.00 | 21.83 | 40.00 | 0.00 | 9.82 | 25.00 | 0.00 | 4.74 | 30.00 | 0.00 | 7.06 |
| *Agrostis stolonifera* | 0.00 | 0.00 | 0.00 | 5.00 | 0.00 | 0.18 | 10.00 | 0.00 | 0.43 | 1.00 | 0.00 | 0.06 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| *Anthoxanthum odoratum* | 40.00 | 0.00 | 14.58 | 20.00 | 1.00 | 6.78 | 50.00 | 1.00 | 22.35 | 30.00 | 1.00 | 11.48 | 50.00 | 4.00 | 22.20 | 10.00 | 1.00 | 2.56 |
| *Bellis perennis* | 0.00 | 0.00 | 0.00 | 1.00 | 0.00 | 0.06 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| *Betonica officinalis* | 5.00 | 0.00 | 0.30 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 1.00 | 0.00 | 0.02 |
| *Bromus hordeaceus* | 0.00 | 0.00 | 0.00 | 4.00 | 0.00 | 0.14 | 0.00 | 0.00 | 0.00 | 1.00 | 0.00 | 0.02 | 1.00 | 0.00 | 0.02 | 1.00 | 0.00 | 0.02 |
| *Carex flacca* | 5.00 | 0.00 | 0.20 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 40.00 | 0.00 | 1.96 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| *Carex* sp. | 1.00 | 0.00 | 0.02 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| *Carex* sp. (receiver) | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 7.00 | 0.00 | 0.44 | 10.00 | 0.00 | 0.41 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| *Centaurea nigra* | 30.00 | 0.00 | 7.50 | 1.00 | 0.00 | 0.08 | 7.00 | 0.00 | 0.56 | 12.00 | 0.00 | 0.30 | 25.00 | 0.00 | 2.32 | 30.00 | 0.00 | 3.28 |
| *Cerastium fontanum* | 1.00 | 0.00 | 0.56 | 1.00 | 0.00 | 0.26 | 7.00 | 0.00 | 1.32 | 2.00 | 0.00 | 0.44 | 2.00 | 0.00 | 0.61 | 1.00 | 0.00 | 0.57 |
| *Cirsium arvense* | 0.00 | 0.00 | 0.00 | 3.00 | 0.00 | 0.62 | 1.00 | 0.00 | 0.13 | 2.00 | 0.00 | 0.09 | 1.00 | 0.00 | 0.09 | 2.00 | 0.00 | 0.06 |
| *Conopodium majus* | 1.00 | 0.00 | 0.46 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| *Crepis capillaris* | 1.00 | 0.00 | 0.04 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| *Cynosurus cristatus* | 1.00 | 0.00 | 0.18 | 10.00 | 0.00 | 2.32 | 20.00 | 0.00 | 4.02 | 25.00 | 0.00 | 2.78 | 25.00 | 0.00 | 5.56 | 50.00 | 1.00 | 19.06 |
| *Dactylis glomerata* | 1.00 | 0.00 | 0.08 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| *Dactylorhiza fuchsii* | 1.00 | 0.00 | 0.16 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 1.00 | 0.00 | 0.06 | 1.00 | 0.00 | 0.02 | 1.00 | 0.00 | 0.02 |
| *Equisteum arvense* | 1.00 | 0.00 | 0.04 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| *Euphrasia* sp. | 2.00 | 0.00 | 1.06 | 1.00 | 0.00 | 0.08 | 15.00 | 0.00 | 1.85 | 30.00 | 0.00 | 5.57 | 40.00 | 0.00 | 6.39 | 20.00 | 0.00 | 1.20 |

(cont.)

| Species | Abundance | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Max | Min | Mean | Max | Min | Mean | Max | Min | Mean | Max | Min | Mean | Max | Min | Mean | Max | Min | Mean |
| | Pi2011 | | | CG2011 | | | CG2012 | | | CG2013 | | | CG2014 | | | CG2015 | | |
| *Festuca rubra* | 5.00 | 0.00 | 1.22 | 10.00 | 0.00 | 2.42 | 30.00 | 0.00 | 7.09 | 50.00 | 0.00 | 9.02 | 40.00 | 0.00 | 11.48 | 60.00 | 1.00 | 16.13 |
| *Galium palustre* | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| *Heracleum sphondylium* | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| *Holcus lanatus* | 15.00 | 0.00 | 3.18 | 15.00 | 0.00 | 3.98 | 30.00 | 0.00 | 3.80 | 20.00 | 0.00 | 3.06 | 30.00 | 1.00 | 8.80 | 40.00 | 2.00 | 17.43 |
| *Hypericum perforatum* | 1.00 | 0.00 | 0.02 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| *Hypochaeris radicata* | 5.00 | 0.00 | 1.34 | 5.00 | 0.00 | 1.28 | 30.00 | 0.00 | 7.50 | 40.00 | 0.00 | 9.87 | 30.00 | 0.00 | 7.65 | 10.00 | 0.00 | 2.26 |
| *Juncus articulatus* | 7.00 | 0.00 | 0.30 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| *Juncus conglomeratus* | 4.00 | 0.00 | 0.14 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| *Juncus effusus* | 0.00 | 0.00 | 0.00 | 10.00 | 0.00 | 0.96 | 4.00 | 0.00 | 0.24 | 15.00 | 0.00 | 1.44 | 1.00 | 0.00 | 0.06 | 2.00 | 0.00 | 0.09 |
| *Lathyrus pratensis* | 1.00 | 0.00 | 0.10 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| *Leontodon hispidus* | 50.00 | 0.00 | 14.04 | 0.00 | 0.00 | 0.00 | 7.00 | 0.00 | 0.67 | 5.00 | 0.00 | 0.65 | 25.00 | 0.00 | 3.11 | 30.00 | 0.00 | 1.33 |
| *Leucanthemum vulgare* | 0.00 | 0.00 | 0.00 | 1.00 | 0.00 | 0.04 | 4.00 | 0.00 | 0.09 | 2.00 | 0.00 | 0.04 | 3.00 | 0.00 | 0.06 | 1.00 | 0.00 | 0.04 |
| *Linum catharticum* | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 1.00 | 0.00 | 0.04 | 1.00 | 0.00 | 0.04 | 0.00 | 0.00 | 0.00 |
| *Lolium perenne* | 0.00 | 0.00 | 0.00 | 1.00 | 0.00 | 0.06 | 2.00 | 0.00 | 0.28 | 4.00 | 0.00 | 0.13 | 1.00 | 0.00 | 0.04 | 1.00 | 0.00 | 0.09 |
| *Lotus corniculatus* | 40.00 | 0.00 | 8.76 | 80.00 | 0.00 | 16.08 | 40.00 | 0.00 | 6.43 | 50.00 | 0.00 | 7.63 | 40.00 | 0.00 | 8.69 | 40.00 | 0.00 | 4.26 |
| *Luzula campestris* | 1.00 | 0.00 | 0.12 | 1.00 | 0.00 | 0.24 | 1.00 | 0.00 | 0.74 | 2.00 | 0.00 | 0.46 | 1.00 | 0.00 | 0.15 | 2.00 | 0.00 | 0.54 |
| *Myosotis arvensis* | 1.00 | 0.00 | 0.02 | 0.00 | 0.00 | 0.00 | 2.00 | 0.00 | 0.15 | 1.00 | 0.00 | 0.15 | 1.00 | 0.00 | 0.17 | 1.00 | 0.00 | 0.24 |
| *Nardus stricta* | 0.00 | 0.00 | 0.00 | 2.00 | 0.00 | 0.06 | 1.00 | 0.00 | 0.02 | 5.00 | 0.00 | 0.09 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| *Plantago lanceolata* | 50.00 | 0.00 | 11.28 | 12.00 | 0.00 | 1.38 | 75.00 | 0.00 | 8.46 | 90.00 | 0.00 | 16.37 | 40.00 | 1.00 | 17.35 | 40.00 | 1.00 | 19.07 |
| *Platanthera chlorantha* | 1.00 | 0.00 | 0.24 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| *Poa pratensis* | 1.00 | 0.00 | 0.14 | 15.00 | 0.00 | 1.92 | 1.00 | 0.00 | 0.15 | 2.00 | 0.00 | 0.09 | 4.00 | 0.00 | 0.22 | 25.00 | 0.00 | 4.30 |
| *Potentilla erecta* | 1.00 | 0.00 | 0.40 | 10.00 | 0.00 | 3.46 | 20.00 | 0.00 | 4.22 | 20.00 | 0.00 | 3.78 | 25.00 | 0.00 | 3.70 | 15.00 | 0.00 | 1.54 |
| *Prunella vulgaris* | 1.00 | 0.00 | 0.02 | 10.00 | 0.00 | 0.96 | 15.00 | 0.00 | 2.35 | 10.00 | 0.00 | 0.93 | 10.00 | 0.00 | 0.93 | 4.00 | 0.00 | 0.91 |
| *Quercus* sp. | 0.00 | 0.00 | 0.00 | 1.00 | 0.00 | 0.02 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 1.00 | 0.00 | 0.02 | 0.00 | 0.00 | 0.00 |
| *Ranunculus acris* | 10.00 | 0.00 | 1.58 | 7.00 | 0.00 | 1.50 | 50.00 | 0.00 | 13.30 | 40.00 | 0.00 | 14.41 | 20.00 | 1.00 | 6.59 | 10.00 | 1.00 | 2.06 |

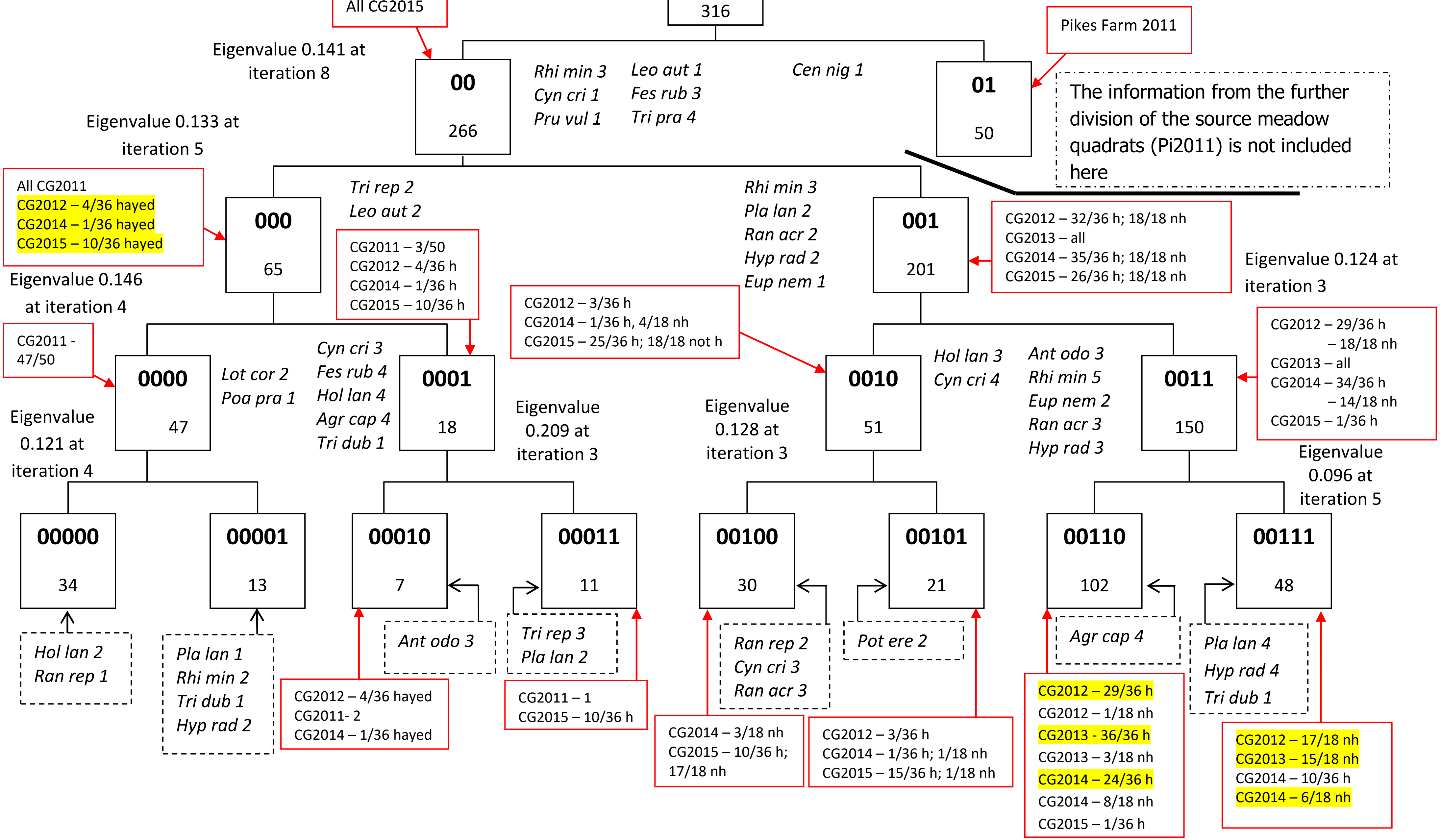
(cont.)

| Species | Abundance | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Max | Min | Mean | Max | Min | Mean | Max | Min | Mean | Max | Min | Mean | Max | Min | Mean | Max | Min | Mean |
| | Pi2011 | | | CG2011 | | | CG2012 | | | CG2013 | | | CG2014 | | | CG2015 | | |
| *Ranunculus bulbosus* | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 1.00 | 0.00 | 0.02 | 4.00 | 0.00 | 0.19 | 1.00 | 0.00 | 0.11 | 2.00 | 0.00 | 0.20 |
| *Ranunculus repens* | 1.00 | 0.00 | 0.04 | 40.00 | 0.00 | 2.16 | 60.00 | 0.00 | 1.72 | 25.00 | 0.00 | 2.76 | 40.00 | 0.00 | 2.00 | 25.00 | 0.00 | 0.94 |
| *Rhinanthus minor* | 7.00 | 0.00 | 1.58 | 25.00 | 0.00 | 4.62 | 60.00 | 4.00 | 28.93 | 90.00 | 0.00 | 50.26 | 30.00 | 2.00 | 12.35 | 30.00 | 1.00 | 10.67 |
| *Rumex acetosa* | 5.00 | 0.00 | 0.48 | 1.00 | 0.00 | 0.16 | 4.00 | 0.00 | 0.32 | 5.00 | 0.00 | 0.57 | 3.00 | 0.00 | 0.44 | 2.00 | 0.00 | 0.43 |
| *Rumex obtusifolius* | 5.00 | 0.00 | 0.12 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| *Scorzoneroides autumnalis* | 1.00 | 0.00 | 0.04 | 10.00 | 1.00 | 3.58 | 10.00 | 0.00 | 1.02 | 4.00 | 0.00 | 0.63 | 2.00 | 0.00 | 0.61 | 7.00 | 0.00 | 1.07 |
| *Silene flos-cuculi* | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| *Stellaria graminea* | 1.00 | 0.00 | 0.06 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| *Taraxacum* spp. | 0.00 | 0.00 | 0.00 | 1.00 | 0.00 | 0.08 | 1.00 | 0.00 | 0.11 | 2.00 | 0.00 | 0.22 | 1.00 | 0.00 | 0.11 | 2.00 | 0.00 | 0.15 |
| *Tragopogon pratensis* | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 1.00 | 0.00 | 0.02 |
| *Trifolium dubium* | 4.00 | 0.00 | 0.38 | 4.00 | 0.00 | 0.44 | 7.00 | 0.00 | 0.78 | 40.00 | 0.00 | 5.15 | 25.00 | 0.00 | 1.94 | 25.00 | 0.00 | 3.04 |
| *Trifolium pratense* | 25.00 | 0.00 | 6.74 | 40.00 | 0.00 | 11.18 | 40.00 | 0.00 | 14.89 | 90.00 | 2.00 | 35.85 | 80.00 | 1.00 | 32.69 | 50.00 | 0.00 | 19.39 |
| *Trifolium repens* | 5.00 | 0.00 | 0.80 | 25.00 | 1.00 | 7.24 | 4.00 | 0.00 | 0.41 | 5.00 | 0.00 | 0.15 | 5.00 | 0.00 | 0.80 | 60.00 | 0.00 | 5.65 |
| *Trisetum flavescens* | 1.00 | 0.00 | 0.26 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 2.00 | 0.00 | 0.06 |

# Appendix 4.2

## Results of TWINSPAN Analysis

Figure 4.1.1: Hierarchical dendrogram of the TWINSPAN analysis for the combined CG2011-CG2015 and Pi2011 data.



# Appendix 5.1
# Golden Field and Three Yew Trees

Table 5.1.1: A comparison of the species and their percentage frequencies, recorded in the source and Golden Field in each year: comparing haying treatment

w/o indicates species seen on a walkover of the field but not recorded in a quadrat
Species in red are species that were transferred by this experiment. Species highlighted in yellow are those that increased in the receiver meadow, after treatment

| | Percentage frequency | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | 2012 | | 2013 | | 2014 | | 2015 | |
| | Hay | No hay | Hay | No hay | Hay | No hay | Hay | No hay |
| ***Agrostis capillaris*** | 48 | 50 | 76 | 78 | 37 | 52 | 50 | 46 |
| *Agrostis stolonifera* | 2 | 4 | 4 | 0 | 0 | 0 | 0 | 6 |
| *Alopecurus geniculatus* | 0 | 0 | 0 | 0 | **2** | 0 | 2 | 4 |
| *Anthoxanthum odoratum* | 100 | 100 | 100 | 98 | 98 | 94 | 100 | 100 |
| *Arrhenatherum elatius* | 2 | 2 | 0 | 0 | 0 | 0 | 0 | 0 |
| ***Bellis perennis*** | 39 | 48 | 56 | **80** | 70 | 81 | 63 | 67 |
| *Betula* seedling | 0 | 0 | 0 | **2** | 0 | **2** | 0 | 0 |
| ***Bromus hordeaceus*** | 41 | 39 | 100 | 94 | 98 | 96 | 96 | 91 |
| *Cardamine pratensis* | **7** | 2 | 4 | 7 | 7 | 6 | 4 | 6 |
| ***Centaurea nigra*** | **9** | 4 | 11 | 9 | 15 | 19 | **28** | 19 |
| ***Cerastium fontanum*** | 76 | 67 | 91 | 83 | 69 | 61 | 100 | 98 |
| *Corylus avellana seedling* | 0 | 0 | 0 | 0 | 0 | 0 | 0 | **2** |
| *Cynosurus cristatus* | 98 | 98 | 100 | 100 | 100 | 100 | 100 | 100 |
| *Dactylis glomerata* | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| ***Dactylorhiza fuchsii*** | 0 | 0 | 0 | 0 | 0 | 0 | 2 | 4 |
| *Euphrasia* sp. | 0 | **2** | **4** | 0 | 6 | 4 | 4 | 4 |
| *Festuca rubra agg.* | 96 | 80 | 67 | 65 | 57 | 54 | 37 | 48 |
| ***Heracleum sphondylium*** | 6 | 6 | 4 | 2 | 2 | 4 | **2** | 0 |
| *Holcus lanatus* | 100 | 100 | 100 | 98 | 100 | 100 | 100 | 100 |
| *Holcus mollis* | 2 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| ***Hypochaeris radicata*** | **22** | 4 | 20 | 19 | 30 | 26 | 46 | 30 |
| *Juncus effusus* | 0 | 0 | 0 | 0 | 0 | **2** | 0 | 0 |
| *Juncus bufonius* | 0 | 0 | 2 | 4 | 4 | 0 | 0 | 0 |
| *Lathyrus pratensis* | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| ***Leontodon hispidus*** | 2 | 2 | 4 | 6 | 7 | 7 | 2 | 4 |
| *Leucanthemum vulgare* | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| *Lolium perenne* | 94 | 96 | 94 | 94 | 85 | 87 | 96 | 89 |
| *Lotus corniculatus* | 0 | 0 | 0 | 0 | 4 | 2 | 2 | 2 |
| *Luzula campestris* | 0 | 0 | 0 | 0 | 0 | 0 | 4 | 2 |
| ***Myosotis arvensis*** | 20 | 20 | 72 | 78 | 30 | 30 | 48 | 50 |
| *Phleum pratense* | 7 | 7 | 44 | 39 | 30 | 31 | 41 | 43 |
| ***Plantago lanceolata*** | **46** | 22 | 59 | 46 | **76** | 59 | **81** | 59 |
| ***Poa pratensis*** | 56 | 44 | 94 | 96 | 78 | 70 | 96 | 85 |

(cont.)

| | Percentage frequency | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | 2012 | | 2013 | | 2014 | | 2015 | |
| | Hay | No hay | Hay | No hay | Hay | No hay | Hay | No hay |
| ***Prunella vulgaris*** | **24** | 11 | **43** | 19 | **70** | 28 | 74 | 61 |
| *Prunus spinosa* seedling | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 2 |
| *Quercus* seedling | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| ***Ranunculus acris*** | 91 | 85 | 93 | 94 | 100 | 94 | 98 | 100 |
| ***Ranunculus bulbosus*** | 0 | 0 | 0 | 0 | 0 | **2** | 9 | 11 |
| *Ranunculus repens* | 98 | 87 | 81 | 87 | 76 | 78 | 89 | 81 |
| *Rhinanthus minor* | 35 | **50** | 56 | 65 | 33 | 20 | 48 | 57 |
| *Rumex acetosa* | 98 | 87 | 96 | 81 | 85 | 69 | **91** | 76 |
| *Rumex crispus* | 0 | 0 | 11 | 9 | 9 | 7 | 6 | **13** |
| ***Rumex obtusifolius*** | 19 | 20 | 2 | 4 | 4 | 4 | **4** | 0 |
| ***Scorzoneroides autumnalis*** | **6** | 0 | 13 | 11 | 9 | **20** | 9 | **17** |
| ***Stellaria graminea*** | 0 | 0 | 0 | 0 | 0 | 0 | **2** | 0 |
| *Taraxacum* spp. | 56 | **81** | **70** | 54 | 63 | 63 | 56 | 44 |
| *Trifolium dubium* | 54 | 52 | 100 | 98 | 98 | 93 | 100 | 100 |
| ***Trifolium pratense*** | **61** | 59 | **63** | 52 | **70** | 59 | **87** | 70 |
| *Trifolium repens* | 22 | 15 | 35 | 28 | 81 | 85 | 76 | 81 |
| ***Trisetum flavescens*** | 0 | 0 | **6** | 0 | 6 | 2 | 0 | 0 |
| *Vicia sativa* | 0 | **2** | **2** | 0 | 2 | 2 | 0 | 0 |
| *Vicia sepium* | 0 | 0 | 0 | 0 | 0 | 0 | 7 | 6 |
| Bare ground | 0 | 0 | **2** | 0 | 4 | 2 | **2** | 0 |

Table 5.1.2: A comparison of the species and their percentage frequencies, recorded in the source and Golden Field in each year: comparing disturbance treatment

w/o indicates species seen on a walkover of the field but not recorded in a quadrat. Figures in red highlights difference between years, Figures highlighted in yellow are 'highest' values; those in blue are 'lowest' values

| | Percentage frequency | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 2012 | | | 2013 | | | 2014 | | | 2015 | | |
| | no-d | low-d | high-d | no-d | low-d | high-d | no-d | low-d | high-d | no-d | low-d | high-d |
| **Species present in post-treatment receiver but not recorded before treatment and were found in the source (i.e. species that were transferred by the treatment in this experiment)** | | | | | | | | | | | | |
| *Dactylorhiza fuchsii* | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | **3** |
| *Heracleum sphondylium* | 6 | **0** | 11 | **0** | 6 | 3 | **0** | 6 | 3 | 0 | 0 | **3** |
| *Leontodon hispidus* | 3 | **0** | 3 | 6 | 6 | 3 | 14 | 8 | **0** | **0** | 3 | 6 |
| *Poa pratensis* | 42 | 50 | 58 | 92 | 97 | 97 | 81 | 61 | 81 | 86 | 97 | 89 |
| *Prunella vulgaris* | 14 | 22 | 17 | 31 | 39 | 22 | 42 | 53 | 53 | 47 | 69 | 81 |
| *Ranunculus bulbosus* | 0 | 0 | 0 | 0 | 0 | 0 | **3** | 0 | 0 | 6 | 8 | 17 |
| *Stellaria graminea* | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 3 | 0 |
| *Trisetum flavescens* | 0 | 0 | 0 | **0** | 3 | 6 | **0** | 8 | 3 | **0** | 0 | 0 |
| **Species present in post-treatment receiver that were not recorded before treatment nor in the source** | | | | | | | | | | | | |
| *Agrostis stolonifera* | **8** | 0 | 0 | **6** | 0 | 0 | 0 | 0 | 0 | **8** | 0 | 0 |
| *Alopecurus geniculatus* | 0 | 0 | 0 | 0 | 0 | 0 | 0 | **3** | 0 | 6 | 3 | 3 |
| *Arrhenatherum elatius* | 0 | 0 | **6** | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| *Betula seedling/sp.* | 0 | 0 | 0 | 0 | 0 | **3** | 0 | **3** | 0 | 0 | 0 | 0 |
| *Cardamine pratensis* | 8 | 6 | **0** | 6 | 11 | **0** | 6 | 8 | 6 | 3 | 6 | 3 |
| *Corylus avellana* seedling | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | **3** |
| *Holcus mollis* | 0 | 0 | **3** | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| *Juncus effusus* | 0 | 0 | 0 | 0 | 0 | 0 | 0 | **3** | 0 | 0 | 0 | 0 |
| *Juncus bufonius* | 0 | 0 | 0 | 3 | **0** | 6 | 3 | **0** | 0 | 0 | **0** | 0 |
| *Prunus spinosa* seedling | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | **3** |

(cont.)

| | Percentage frequency | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 2012 | | | 2013 | | | 2014 | | | 2015 | | |
| | no-d | low-d | high-d | no-d | low-d | high-d | no-d | low-d | high-d | no-d | low-d | high-d |
| *Rumex crispus* | 0 | 0 | 0 | 3 | 11 | 17 | 3 | 14 | 8 | 3 | 6 | 17 |
| *Vicia sativa* | **3** | 0 | 0 | **3** | 0 | 0 | 3 | **0** | 3 | 0 | 0 | 0 |
| *Vicia sepium* | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 6 | 8 | 6 |
| *(Bare ground)* | 0 | **3** | 0 | 0 | 0 | **3** | 6 | **0** | 3 | **3** | 0 | 0 |
| **Species that increased substantially post-treatment** | | | | | | | | | | | | |
| *Agrostis capillaris* | 50 | 47 | 50 | 78 | 78 | 75 | 47 | 44 | 42 | 47 | 44 | 53 |
| *Bromus hordeaceus* | 28 | 47 | 44 | 97 | 100 | 94 | 100 | 94 | 97 | 100 | 86 | 94 |
| *Centaurea nigra* | 3 | 8 | 8 | 6 | 11 | 14 | 14 | 22 | 14 | 8 | 22 | 36 |
| *Cerastium fontanum* | 72 | 69 | 72 | 86 | 89 | 86 | 67 | 67 | 61 | 100 | 100 | 97 |
| *Hypochaeris radicata* | 8 | 17 | 14 | 3 | 36 | 19 | 19 | 25 | 39 | 33 | 39 | 39 |
| *Scorzoneroides autumnalis* | 6 | **0** | 3 | 11 | 17 | 8 | 14 | 19 | 11 | 8 | 19 | 11 |
| *Plantago lanceolata* | 33 | 36 | 33 | 47 | 64 | 47 | 56 | 75 | 72 | 58 | 75 | 72 |
| *Ranunculus acris* | 83 | 92 | 89 | 89 | 92 | 100 | 97 | 100 | 94 | 97 | 100 | 100 |
| *Trifolium pratense* | 47 | 61 | 72 | 50 | 64 | 58 | 58 | 75 | 61 | 61 | 89 | 83 |
| *Bellis perennis** | 36 | 47 | 47 | 64 | 67 | 72 | 69 | 81 | 78 | 58 | 69 | 67 |
| *Myosotis arvensis** | 17 | 17 | 28 | 67 | 72 | 86 | 28 | 47 | 14 | 39 | 53 | 50 |
| *Rumex obtusifolius** | 19 | 14 | 25 | 6 | 3 | **0** | **0** | 6 | 6 | 3 | **0** | 3 |
| **These last three species were not in the source meadow* | | | | | | | | | | | | |
| **Species that decreased substantially post-treatment** | | | | | | | | | | | | |
| *Euphrasia* sp. | 0 | 0 | **3** | 0 | 0 | **6** | 8 | 6 | **0** | **0** | 6 | 6 |
| *Festuca rubra* | 89 | 83 | 92 | 61 | 67 | 69 | 69 | 56 | 42 | 47 | 42 | 44 |
| *Lotus corniculatus* | 0 | 0 | 0 | 0 | 0 | 0 | **8** | 0 | 0 | 0 | 0 | **6** |
| *Luzula campestris* | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 3 | 3 | 3 |

(cont.)

| | Percentage frequency | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 2012 | | | 2013 | | | 2014 | | | 2015 | | |
| | no-d | low-d | high-d | no-d | low-d | high-d | no-d | low-d | high-d | no-d | low-d | high-d |
| *Phleum pratense** | 8 | 3 | 11 | 31 | 44 | 50 | 31 | 22 | 39 | 33 | 39 | 50 |
| *Quercus seedling/sp* | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| *Rhinanthus minor* | 47 | 44 | 36 | 67 | 64 | 50 | 42 | 19 | 19 | 64 | 53 | 44 |
| *Trifolium repens* | 28 | 11 | 17 | 36 | 31 | 28 | 81 | 81 | 89 | 86 | 75 | 75 |
| *Species was not in the source meadow | | | | | | | | | | | | |
| **Species which did not change in frequency substantially** | | | | | | | | | | | | |
| *Anthoxanthum odoratum* | 100 | 100 | 100 | 100 | 100 | 97 | 92 | 100 | 97 | 100 | 100 | 100 |
| *Cynosurus cristatus* | 100 | 97 | 97 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 |
| *Holcus lanatus* | 100 | 100 | 100 | 100 | 100 | 97 | 100 | 100 | 100 | 100 | 100 | 100 |
| *Lolium perenne* | 94 | 97 | 94 | 94 | 94 | 94 | 86 | 81 | 92 | 83 | 97 | 97 |
| *Rumex acetosa* | 83 | 97 | 97 | 81 | 97 | 89 | 69 | 83 | 78 | 72 | 89 | 83 |
| *Taraxacum* spp. | 67 | 69 | 69 | 61 | 67 | 58 | 56 | 64 | 69 | 36 | 58 | 53 |
| *Ranunculus repens* | 100 | 83 | 94 | 86 | 83 | 83 | 72 | 75 | 83 | 86 | 81 | 89 |
| *Trifolium dubium* | 50 | 50 | 58 | 100 | 97 | 100 | 92 | 100 | 94 | 100 | 100 | 100 |
| *The last two species were not in the source meadow | | | | | | | | | | | | |

Table 5.1.3: A comparison of the species and their percentage frequencies, recorded in the source and Golden Field in each year: comparing grazing treatment

w/o indicates species seen on a walkover of the field but not recorded in a quadrat

| | **Percentage frequency** | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | **2012** | | **2013** | | **2014** | | **2015** | |
| | **Grazed** | **Not grazed** | **Grazed** | **Not grazed** | **Grazed** | **Not grazed** | **Grazed** | **Not grazed** |
| *Agrostis capillaris* | 0 | **98** | 69 | **85** | 20 | **69** | 13 | **83** |
| *Agrostis stolonifera* | 0 | **6** | 2 | 2 | 0 | 0 | 2 | 4 |
| *Alopecurus geniculatus* | 0 | 0 | 0 | 0 | 2 | 0 | 4 | 2 |
| *Anthoxanthum odoratum* | 100 | 100 | 98 | 100 | 96 | 96 | 100 | 100 |
| *Arrhenatherum elatius* | 0 | **4** | 0 | 0 | 0 | 0 | 0 | 0 |
| *Bellis perennis* | **87** | 0 | **89** | 46 | **98** | 54 | **98** | 31 |
| *Betula* seedling | 0 | 0 | **2** | 0 | 0 | **2** | 0 | 0 |
| *Bromus hordeaceus* | 39 | 41 | 98 | 96 | 100 | 94 | 98 | 89 |
| *Cardamine pratensis* | **7** | 2 | **9** | 2 | **13** | 0 | **9** | 0 |
| *Centaurea nigra* | 4 | 9 | 4 | **17** | 13 | 20 | 24 | 22 |
| *Cerastium fontanum* | **91** | 52 | 85 | 89 | 61 | 69 | 98 | 100 |
| *Corylus avellana* seedling | 0 | 0 | 0 | 0 | 0 | 0 | 0 | **2** |
| *Cynosurus cristatus* | 100 | 96 | 100 | 100 | 100 | 100 | 100 | 100 |
| *Dactylis glomerata* | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| *Dactylorhiza fuchsii* | 0 | 0 | 0 | 0 | 0 | 0 | **6** | 0 |
| *Euphrasia* sp. | **2** | 0 | **4** | 0 | 2 | **7** | 2 | **6** |
| *Festuca rubra agg.* | 100 | 76 | 35 | 96 | 28 | 83 | 31 | 54 |
| *Heracleum sphondylium* | 0 | **11** | 0 | **6** | 0 | **6** | 0 | **2** |
| *Holcus lanatus* | 100 | 100 | 98 | 100 | 100 | 100 | 100 | 100 |
| *Holcus mollis* | 0 | **2** | 0 | 0 | 0 | 0 | 0 | 0 |
| *Hypochaeris radicata* | 17 | 9 | 9 | 30 | 20 | 35 | 26 | 50 |
| *Juncus effusus* | 0 | 0 | 0 | 0 | 2 | 0 | 0 | 0 |
| *Juncus bufonius* | 0 | 0 | 2 | 4 | 4 | 0 | 0 | 0 |
| *Lathyrus pratensis* | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| *Leontodon hispidus* | 0 | **4** | 0 | **9** | 0 | **15** | 0 | **6** |
| *Leucanthemum vulgare* | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| *Lolium perenne* | 93 | 98 | 96 | 93 | 94 | 78 | 96 | 89 |
| *Lotus corniculatus* | 0 | 0 | 0 | 0 | 0 | **6** | 2 | 2 |
| *Luzula campestris* | 0 | 0 | 0 | 0 | 0 | 0 | 2 | 4 |
| *Myosotis arvensis* | **41** | 0 | 74 | 76 | 15 | **44** | **65** | 33 |
| *Phleum pratense* | 9 | 6 | 70 | 13 | 59 | 2 | 74 | 9 |
| *Plantago lanceolata* | 31 | 37 | 43 | **63** | 54 | **81** | 50 | **91** |
| *Poa pratensis* | 89 | 11 | 100 | 91 | 70 | 78 | 100 | 81 |
| *Prunella vulgaris* | 19 | 17 | 31 | 30 | 48 | 50 | 59 | 76 |
| *Prunus spinosa* seedling | 0 | 0 | 0 | 0 | 0 | 0 | 0 | **2** |
| *Quercus* seedling | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| *Ranunculus acris* | 78 | **98** | 87 | **100** | 94 | 100 | 98 | 100 |

(cont.)

| | Percentage frequency | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | 2012 | | 2013 | | 2014 | | 2015 | |
| | **Grazed** | **Not grazed** | **Grazed** | **Not grazed** | **Grazed** | **Not grazed** | **Grazed** | **Not grazed** |
| *Ranunculus bulbosus* | 0 | 0 | 96 | 72 | 0 | **2** | 2 | **19** |
| *Ranunculus repens* | 98 | 87 | 46 | **74** | **98** | 56 | **100** | 70 |
| *Rhinanthus minor* | 44 | 41 | 81 | 96 | 7 | **46** | 24 | **81** |
| *Rumex acetosa* | 89 | 96 | 9 | 11 | 59 | 94 | 76 | 91 |
| *Rumex crispus* | 0 | 0 | 0 | 0 | **15** | 2 | **15** | 4 |
| *Rumex obtusifolius* | 20 | 19 | 2 | 4 | 4 | 4 | 0 | **4** |
| *Scorzoneroides autumnalis* | 0 | **6** | 11 | 13 | 15 | 15 | **17** | 9 |
| *Stellaria graminea* | 0 | 0 | 0 | 0 | 0 | 0 | **2** | 0 |
| *Taraxacum* spp. | 78 | 59 | 43 | **81** | 50 | **76** | 35 | **65** |
| *Trifolium dubium* | **100** | 6 | 100 | 98 | 96 | 94 | 100 | 100 |
| *Trifolium pratense* | **70** | 50 | 37 | **78** | 50 | **80** | 65 | **93** |
| *Trifolium repens* | 20 | 17 | 37 | 26 | **93** | 74 | 98 | 59 |
| *Trisetum flavescens* | 0 | 0 | 0 | **6** | 0 | **7** | 0 | 0 |
| *Vicia sativa* | 0 | **2** | 0 | **2** | 0 | 0 | 0 | 0 |
| *Vicia sepium* | 0 | 0 | 0 | 0 | 0 | **4** | 0 | **9** |
| Bare ground | **2** | 0 | **2** | 0 | **6** | 0 | **2** | 0 |

## Appendix 5.2

## Comparison of mean number of species per quadrat and total number of species per meadow

As might be expected, of all the groups, 3YT had the highest mean number of species per quadrat and the highest total number of species.

GF2012all had a lower mean number of species than GF2011all, however all the other post-treatment years had a higher mean number of species than GF2011all. All the post-treatment years for the 'all' group had a higher total number of species than GF2011all.

The combined hayed group had a higher mean number of species per quadrat than the not-hayed group in every year. The combined hayed group had the same total number of species in two of the years, higher in another year and lower in the remaining year.

All the post-treatment years for the combined not-grazed-all quadrats group had a higher mean number of species per quadrat than the grazed-all quadrats group, except for the first year (2012). All the post-treatment years for the not-grazed group had more species than the grazed group.

The not-grazed group had more species per quadrat compared to their grazed counterpart in 19 out of the 24 categories. The notable exception being in the first post-treatment year, when the grazed group counterpart had the higher number. The not-grazed categories again had more species compared to their grazed counterparts in 19 out of the 28 categories and in three other categories they had an equal number.

The highest mean number of species per quadrat recorded was that of high-disturbance/hayed/not-grazed in 2015. The highest mean number in the grazed portion was in low-disturbance/hayed, again in 2015.  The highest total number of species was found in high-disturbance/hayed/not-grazed in 2015, equal with high-disturbance/not-hayed/not-grazed/2015. The highest in the not-grazed portion was in low-disturbance/hayed/2015.

Of the hayed and not-hayed groups: hayed had a higher total number of species in 12 cases, not-hayed in seven and they had equal numbers in five cases (e.g. in 2012 no-disturbance/hayed/grazed had a higher total number than no-disturbance/not-hayed/grazed). Hayed had the higher mean number of species per quadrat in 19 cases, not-hayed in four and they were equal once.

There does not seem to be a trend in the disturbance figures: no-disturbance, low-disturbance and high-disturbance all have the highest figures for mean and/or total number of species in various cases and the number of species does not appear to increase (or decrease) from no-disturbance to high-disturbance when considering the figures for the treatment strips (i.e. disturbance, grazing and haying). When combining the grazed and not-grazed data, however, there does seem to more of a trend of increasing total and mean numbers of species from no-disturbance to high-disturbance.

Table 5.2.1: Mean number of species per quadrat for Golden Field and its source in the quadrat surveys

| Meadow/treatment | | Year of survey | | | | |
|---|---|---|---|---|---|---|
| | | 2011 | 2012 | 2013 | 2014 | 2015 |
| Source meadow(3YT) | | 16.00 | N/A | | | |
| Receiver(GF): | | | | | | |
| All quadrats | | 14.12 | 13.92 | 17.38 | 16.61 | 17.98 |
| All quadrats/grazed | Grazed | N/A | 15.26 | 16.69 | 15.81 | 17.91 |
| No-d/not-hayed | | | 13.78 | 14.56 | 14.56 | 15.33 |
| Low-d/not-hayed | | | 15.22 | 17.33 | 15.89 | 17.44 |
| High-d/not-hayed | | | 16.00 | 15.78 | 15.33 | 18.44 |
| No-d/hayed | | | 15.33 | 17.00 | 15.89 | 17.11 |
| Low-d/hayed | | | 16.00 | 18.11 | 16.78 | 19.00 |
| High-d/hayed | | | 15.22 | 17.33 | 16.33 | 16.11 |
| All quadrats/not-grazed | Not-grazed | N/A | 12.57 | 18.07 | 17.43 | 18.41 |
| No-d/not-hayed | | | 12.00 | 17.33 | 16.11 | 16.33 |
| Low-d/not-hayed | | | 11.00 | 19.11 | 17.78 | 18.89 |
| High-d/not-hayed | | | 12.78 | 17.89 | 17.11 | 18.67 |
| No-d/hayed | | | 13.00 | 17.78 | 18.56 | 18.78 |
| Low-d/hayed | | | 12.56 | 17.78 | 17.89 | 18.33 |
| High-d/hayed | | | 14.11 | 18.56 | 17.11 | 19.67 |
| No-d/not-hayed | All | N/A | 12.89 | | | 15.83 |
| Low-d/not-hayed | | | 13.11 | | | 18.17 |
| High-d/not-hayed | | | 14.39 | | | 18.56 |
| No-d/hayed | | | 14.17 | | | 17.94 |
| Low-d/hayed | | | 14.28 | | | 18.67 |
| High-d/hayed | | | 14.67 | | | 19.17 |
| Hayed | | | 14.37 | 17.76 | 17.11 | 18.59 |
| Not-hayed | | | 13.46 | 17.00 | 16.13 | 17.76 |

Table 5.2.2: Total number of species for Golden Field and its source in the quadrat surveys

| | | 2011 | 2012 | 2013 | 2014 | 2015 |
|---|---|---|---|---|---|---|
| Source meadow(3YT) | | 41 | N/A | | | |
| Receiver(GF): | | | | | | |
| All quadrats | | 28 | 34 | 36 | 39 | 40 |
| All quadrats/grazed | Grazed | N/A | 26 | 32 | 32 | 35 |
| No-d/not-hayed | | | 21 | 24 | 24 | 22 |
| Low-d/not-hayed | | | 23 | 26 | 26 | 27 |
| High-d/not-hayed | | | 23 | 26 | 24 | 29 |
| No-d/hayed | | | 23 | 28 | 27 | 25 |
| Low-d/hayed | | | 24 | 26 | 28 | 30 |
| High-d/hayed | | | 22 | 26 | 23 | 27 |
| All quadrats/not-grazed | Not-grazed | N/A | 31 | 34 | 35 | 38 |
| No-d/not-hayed | | | 20 | 26 | 28 | 26 |
| Low-d/not-hayed | | | 18 | 27 | 29 | 29 |
| High-d/not-hayed | | | 23 | 26 | 26 | 31 |
| No-d/hayed | | | 23 | 24 | 28 | 28 |
| Low-d/hayed | | | 20 | 27 | 28 | 25 |
| High-d/hayed | | | 26 | 28 | 25 | 31 |
| No-d/not-hayed | All | N/A | 25 | | | 27 |
| Low-d/not-hayed | | | 24 | | | 32 |
| High-d/not-hayed | | | 28 | | | 35 |
| No-d/hayed | | | 29 | | | 31 |
| Low-d/hayed | | | 26 | | | 32 |
| High-d/hayed | | | 29 | | | 35 |
| Hayed | | | 32 | 35 | 36 | 38 |
| Not-hayed | | | 32 | 32 | 37 | 38 |

## Appendix 5.3

## Species Diversity Measures

GF2013 not-grazed had the highest diversity as given by Simpson's Index, however, GF2014 not-grazed had the lowest (Table 5.3.1). 3YT2011 had lower diversity than GF2011. Of the post-treatment groups, the hayed quadrats had higher diversity than the not-hayed group in all years, except 2014 while the not-grazed group had higher diversity than the grazed group, again in all years except 2014.

GF2013 not-hayed had the highest evenness and GF2014 not-grazed had the lowest evenness. 3YT had lower evenness than GF2011. The grazed group had higher evenness than not-grazed in two years, while the reverse was true in the remaining two. The hayed group had an equal evenness with the not-hayed group in 3 years and a slightly lower evenness in one year (2013). The evenness was generally lower after treatment than before treatment.

Of the treatment groups, GF2015 not-hayed and GF2015 not-grazed had the equal highest dominance, the most dominant species being *Trifolium dubium* in both cases. The Berger-Parker Index, however, suggests that 3YT has the highest dominance of all these groups – the most dominant species being *Leontodon hispidus*. GF2011 had a lower dominance than 3YT, although the most dominant species was less desirable (*Ranunculus repens*). The group of quadrats with the least dominance, according to this Index, was GF2013, not-grazed (*T. dubium*). The grazed group had a higher dominance than GF not-grazed in two years and the reverse was true in the remaining two years. The hayed group had a higher dominance in 2 years, an equal figure in 1 year and a lower dominance in 1 year. In any year, both hayed and not-hayed groups had the same species as the most dominant.

Table 5.3.1: Species diversity measures for Golden Field and its source

| | **Simpson's Index** | **Simpson's Measure of Evenness** | **Berger-Parker** | **Species with highest total % cover** |
|---|---|---|---|---|
| | | | | |
| 3YT2011 | 1.80 | 0.15 | 0.35 | *Leontodon hispidus* |
| GF2011 | 1.92 | 0.24 | 0.23 | *Ranunculus repens* |
| | | | | |
| **2012** | | | | |
| All | 2.05 | 0.24 | 0.19 | *Anthoxanthum odoratum* |
| Hayed | 2.05 | 0.24 | 0.19 | *Anthoxanthum odoratum* |
| Not-hayed | 2.03 | 0.24 | 0.19 | *Anthoxanthum odoratum* |
| Grazed | 1.74 | 0.22 | 0.31 | *Trifolium dubium* |
| Not-grazed | 1.87 | 0.21 | 0.26 | *Holcus lanatus* |
| | | | | |
| **2013** | | | | |
| All | 1.90 | 0.16 | 0.26 | *Ranunculus repens* |
| Hayed | 2.32 | 0.27 | 0.19 | *Ranunculus repens* |
| Not-hayed | 2.18 | 0.28 | 0.18 | *Ranunculus repens* |
| Grazed | 1.72 | 0.18 | 0.30 | *Ranunculus repens* |
| Not-grazed | 2.44 | 0.26 | 0.16 | *Trifolium dubium* |
| | | | | |
| **2014** | | | | |
| All | 1.91 | 0.17 | 0.29 | *Trifolium dubium* |
| Hayed | 1.90 | 0.18 | 0.30 | *Trifolium dubium* |
| Not-hayed | 1.91 | 0.18 | 0.28 | *Trifolium dubium* |
| Grazed | 2.01 | 0.23 | 0.23 | *Cynosurus cristatus* |
| Not-grazed | 1.49 | 0.13 | 0.44 | *Trifolium dubium* |
| | | | | |
| **2015** | | | | |
| All | 1.87 | 0.16 | 0.31 | *Trifolium dubium* |
| Hayed | 1.89 | 0.17 | 0.30 | *Trifolium dubium* |
| Not-hayed | 1.85 | 0.17 | 0.33 | *Trifolium dubium* |
| Grazed | 1.65 | 0.15 | 0.30 | *Ranunculus repens* |
| Not-grazed | 1.91 | 0.18 | 0.33 | *Trifolium dubium* |

## Appendix 5.4

## Comparison with the NVC

3YT most closely matched MG5 (co-efficient of 63.83). All of the Golden Field datasets matched MG6b most closely – the least similar being the GF2012 high-disturbance/not-hayed/grazed group of quadrats and the closest match being the GF2012 hayed group. The closest match of the treatment groups was GF2015 no-disturbance/not-hayed/not-grazed.

Of MG5 community matches (Table 5.4.2), the GF2014 not-grazed group has the highest co-efficient for a match (MG5a:66.18, the group's 2$^{nd}$ highest match). Of the treatment groups, GF2014/high-disturbance/hayed/not-grazed had the closest match to an MG5 community and GF2012/no-disturbance/not-hayed/not-grazed had the lowest (no match). Through the years, not-grazed always had a closer match to an MG5 group than grazed and hayed a closer match than not-hayed in three out of four cases. Of the MG5 communities, all the GF groups matched MG5a most closely.

Table 5.4.1: Co-efficients of similarity from MAVIS, highest result
All GF groups match most closely with MG6b. Figures in red are lower than the previous year's match

| | **2011** | **2012** | **2013** | **2014** | **2015** |
|---|---|---|---|---|---|
| Golden Field | | | | | |
| All | 66.23 | 70.18 | **67.80** | 69.89 | **67.09** |
| Hayed | | 73.57 | **68.82** | 68.86 | **65.74** |
| Not-hayed | | 70.24 | **67.76** | 71.32 | **69.11** |
| Grazed | | 66.71 | 67.45 | 71.55 | **67.78** |
| Not-grazed | | 71.21 | **69.85** | **68.10** | **68.07** |
| No-d/not-hayed/grazed | | 66.13 | 66.63 | 70.55 | **66.67** |
| Low-d/not-hayed/grazed | | 63.91 | 65.93 | 69.28 | **68.13** |
| High-d/not-hayed/grazed | | 63.45 | 67.74 | 70.55 | **67.72** |
| No-d/hayed/grazed | | 67.02 | 67.40 | 67.43 | **67.00** |
| Low-d/hayed/grazed | | 68.60 | **66.67** | 68.93 | **67.05** |
| High-d/hayed/grazed | | 64.59 | 68.47 | 65.86 | 65.91 |
| No-d/not-hayed/not-grazed | | 70.96 | **69.61** | 72.64 | 72.69 |
| Low-d/not-hayed/not-grazed | | 64.30 | 68.75 | 68.44 | **66.63** |
| High-d/not-hayed/not-grazed | | 67.91 | **63.35** | 71.07 | **68.05** |
| No-d/hay/not-grazed | | 72.52 | **67.00** | **65.87** | 67.38 |
| Low-d/hay/not-grazed | | 69.99 | **68.07** | 69.19 | **67.69** |
| High-d/hay/not-grazed | | 71.86 | **67.02** | 68.85 | **66.29** |
| No-d all | | 72.66 | **68.86** | 69.57 | 73.13 |
| No-d/hayed | | 72.98 | **68.49** | | |
| No-d/not-hayed | | 71.60 | **68.58** | | |
| No-d/grazed | | 66.63 | 66.67 | | |
| No-d/not-grazed | | 72.15 | **70.31** | | |
| Low-d all | | 70.28 | **68.82** | 70.25 | **68.08** |
| Low-d/hayed | | 72.23 | **70.60** | | |
| Low-d/not-hayed | | 69.14 | **68.09** | | |
| Low-d/grazed | | 67.09 | **67.06** | | |
| Low-d/not-grazed | | 68.78 | 70.19 | | |
| High-d all | | 69.40 | **67.38** | 71.43 | **66.40** |
| High-d/hayed | | 71.31 | **68.45** | | |
| High-d/not-hayed | | 69.05 | **67.00** | | |
| High-d/grazed | | 64.67 | 69.24 | | |
| High-d/not-grazed | | 67.76 | **67.02** | | |
| 3YT top match (MG5) | 63.83 | | | | |
| 3YT 2nd match (MG5a) | 63.64 | | | | |
| 3YT 3rd match (MG5b) | 61.83 | | | | |
| 3YT 4th match (MG5c) | 61.49 | | | | |

Table 5.4.2 Closest matches to an MG5 community type
(NB All GF groups match closest to MG5a). Figures in red are higher than in the previous year

| | 2011 | 2012 | 2013 | 2014 | 2015 |
|---|---|---|---|---|---|
| Golden Field | | | | | |
| All | 53.60 | 60.41 | 57.51 | **62.85** | 61.35 |
| Hayed | | 63.16 | 60.04 | **61.05** | **61.83** |
| Not-hayed | | 58.47 | 56.99 | **61.94** | 60.13 |
| Grazed | | 54.48 | 54.03 | **57.27** | **58.00** |
| Not-grazed | | 57.83 | **60.89** | **66.18** | 62.09 |
| No-d/not-hayed/grazed | | 54.69 | 51.53 | **56.85** | 49.15 |
| Low-d/not-hayed/grazed | | 51.96 | **53.19** | **55.08** | **56.54** |
| High-d/not-hayed/grazed | | 51.23 | **53.96** | **57.09** | **59.33** |
| No-d/hayed/grazed | | 54.28 | **54.99** | **56.72** | 55.46 |
| Low-d/hayed/grazed | | 58.44 | 54.72 | **57.50** | 57.14 |
| High-d/hayed/grazed | | 51.96 | **56.07** | 54.23 | 55.09 |
| No-d/not-hayed/not-grazed | | None | **57.22** | **62.78** | 60.74 |
| Low-d/not-hayed/not-grazed | | 49.53 | **62.82** | **65.26** | 61.88 |
| High-d/not-hayed/not-grazed | | 55.33 | 54.70 | **60.8** | 59.33 |
| No-d/hay/not-grazed | | 58.24 | 56.58 | **62.94** | 60.95 |
| Low-d/hay/not-grazed | | 55.33 | **61.46** | **62.61** | 60.85 |
| High-d/hay/not-grazed | | 62.26 | 61.55 | **63.73** | 62.77 |
| No-d all | | 59.83 | 57.02 | **60.63** | 59.87 |
| No-d/hayed | | 60.21 | 58.13 | | |
| No-d/not-hayed | | 58.37 | 54.40 | | |
| No-d/grazed | | 54.62 | 53.27 | | |
| No-d/not-grazed | | 56.30 | **58.00** | | |
| Low-d all | | 58.88 | **59.93** | **61.41** | 60.86 |
| Low-d/hayed | | 61.44 | 59.59 | | |
| Low-d/not-hayed | | 55.69 | **59.59** | | |
| Low-d/grazed | | 54.73 | 54.48 | | |
| Low-d/not-grazed | | 53.39 | **62.5** | | |
| High-d all | | 59.62 | 57.47 | **60.90** | 60.76 |
| High-d/hayed | | 60.27 | 60.08 | | |
| High-d/not-hayed | | 58.85 | 54.94 | | |
| High-d/grazed | | 52.41 | **53.88** | | |
| High-d/not-grazed | | 59.96 | 59.05 | | |
| 3YT top match (MG5) | 63.83 | | | | |
| 3YT 2nd match (MG5a) | 63.64 | | | | |
| 3YT 3rd match (MG5b) | 61.83 | | | | |
| 3YT 4th match (MG5c) | 61.49 | | | | |

**Appendix 5.5**

**PCA analyses**

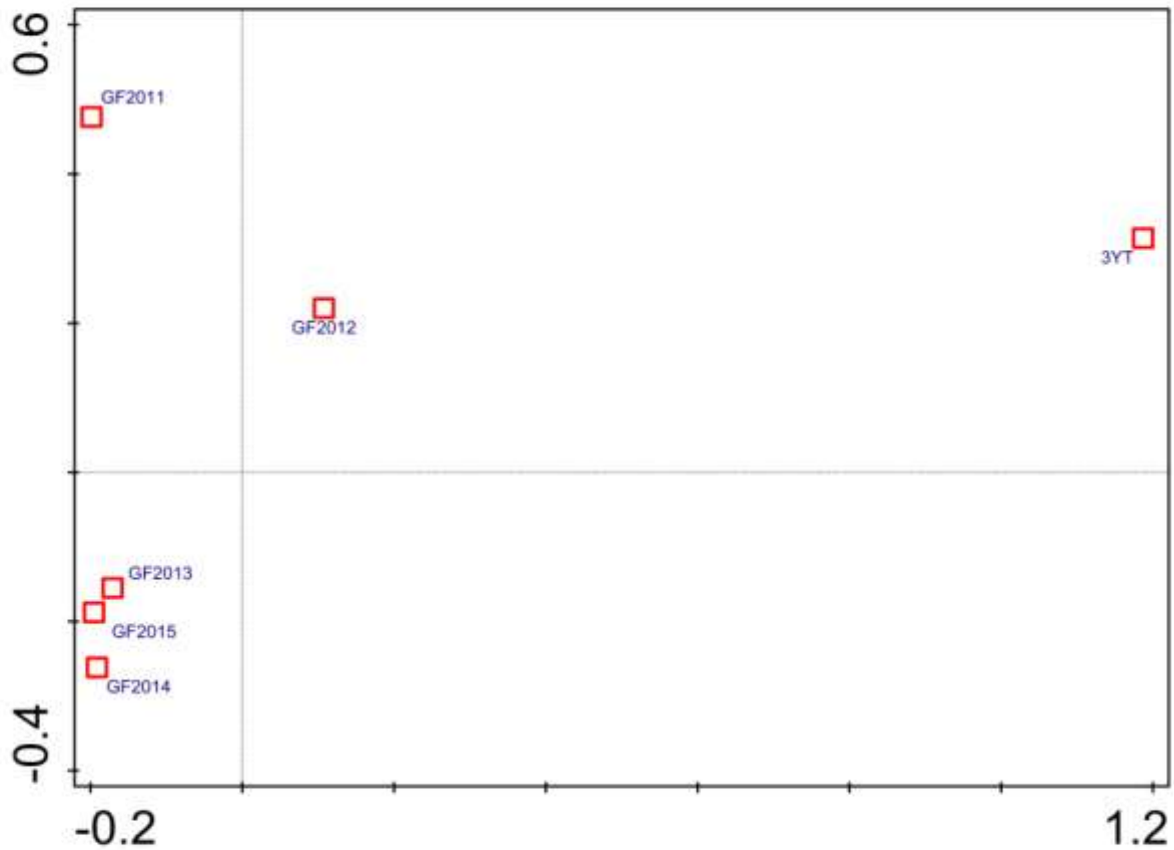



Figure 5.5.1: PCA ordination plot with centroids for years.

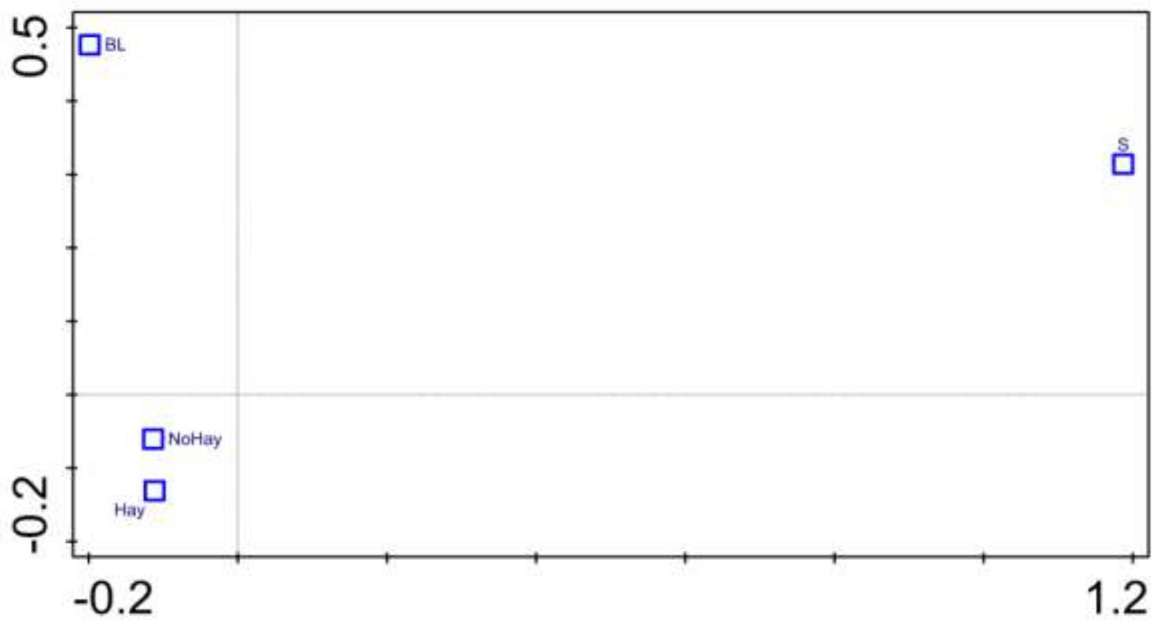



Figure 5.5.2: PCA ordination plot with centroids for haying.
BL is the baseline (i.e. GF2011); S is the source meadow (3YT2011).

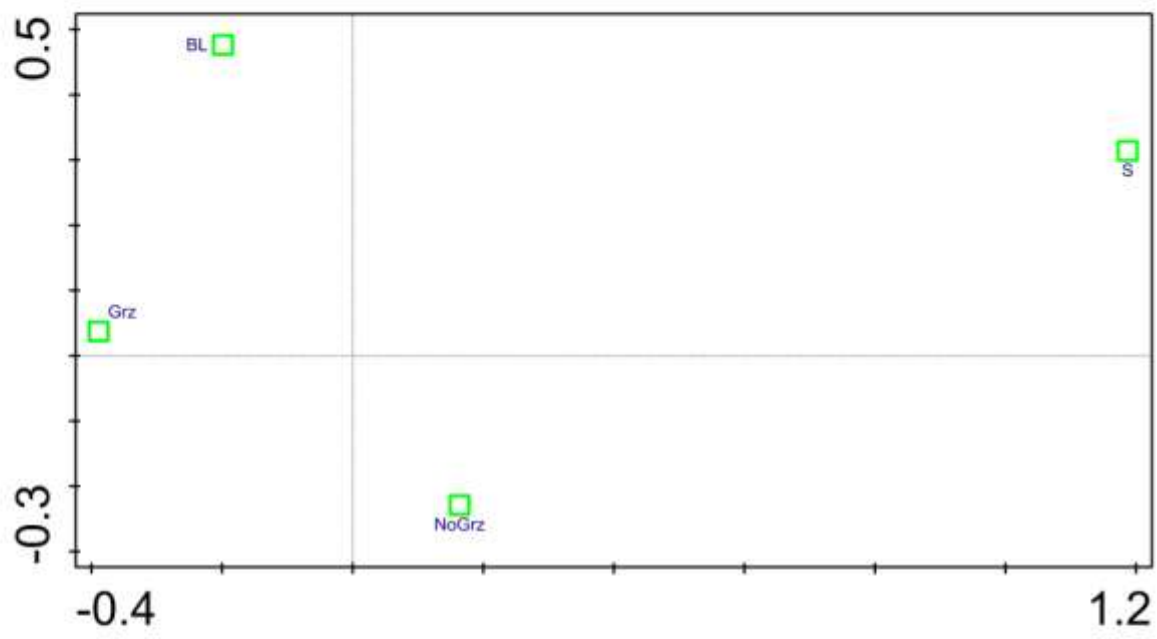



Figure 5.5.3: PCA ordination plot with centroids for grazing.
BL is the baseline (i.e. GF2011); S is the source (3YT2011); Grz is the grazed quadrats, NoGrz is the not-grazed quadrats.

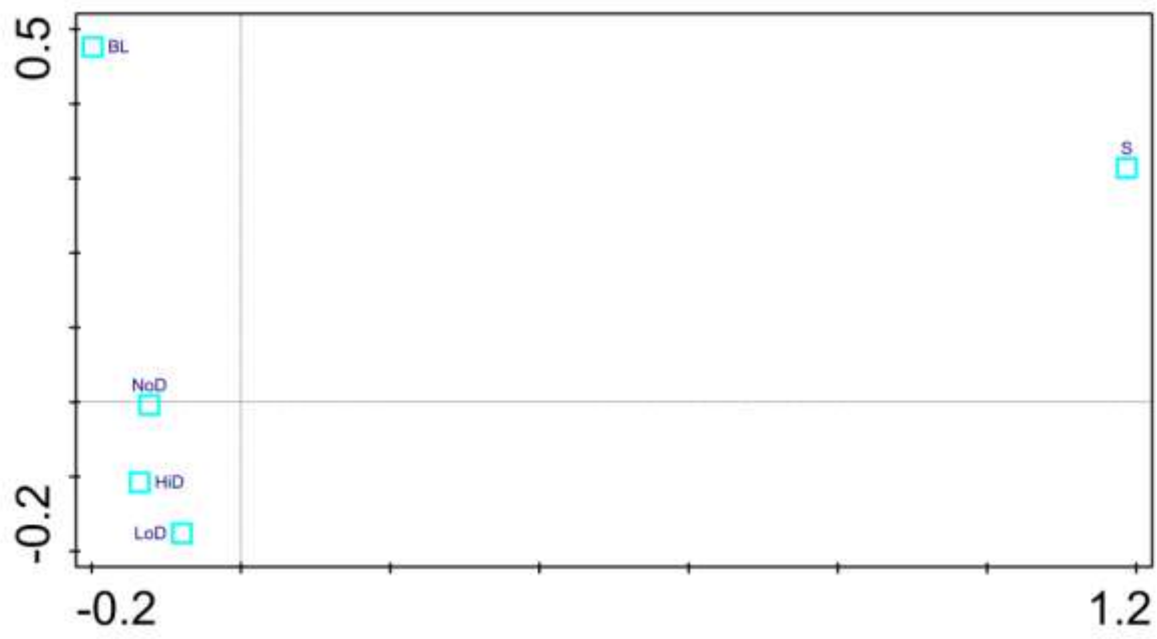



Figure 5.5.4: PCA ordination plot with centroids for disturbance.
BL is the baseline (i.e. GF2011); S is the source (3YT2011); NoD is No-disturbance; LoD is Low-disturbance; HiD is High-disturbance.

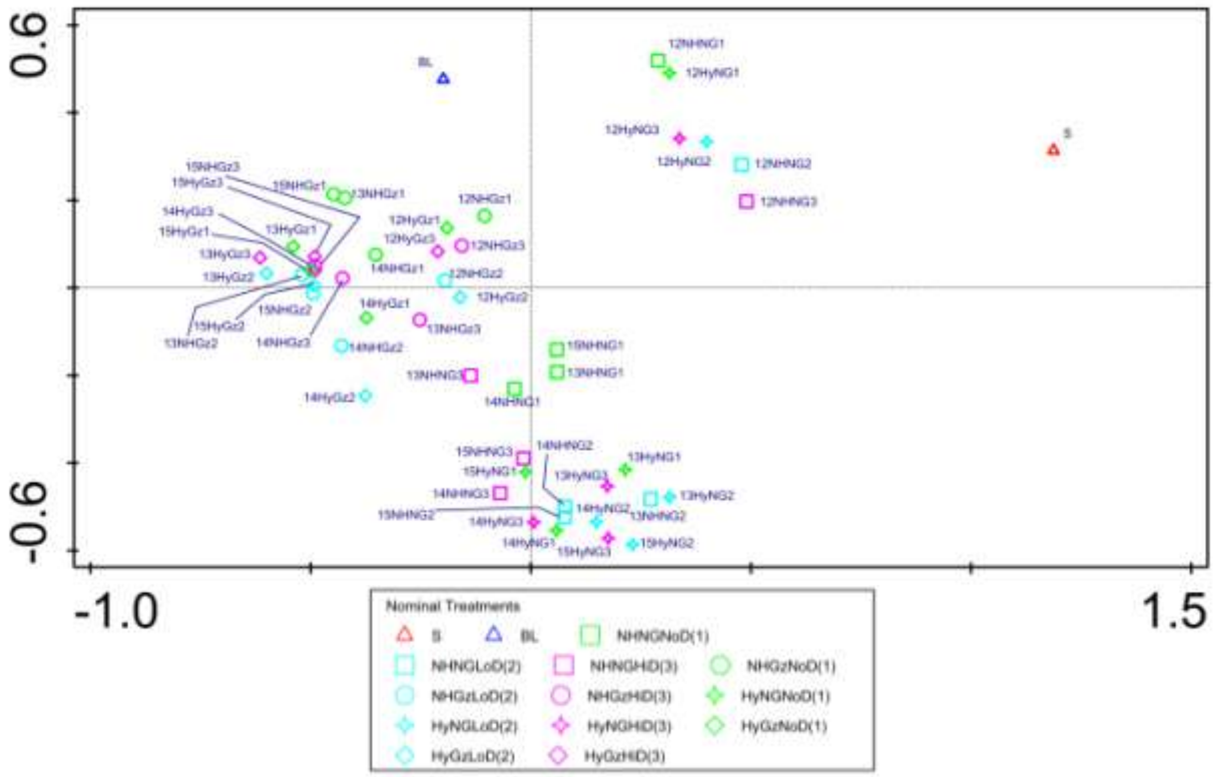



Figure 5.5.5: PCA samples plot of GF source and receiver with samples shown centroids for all treatments.

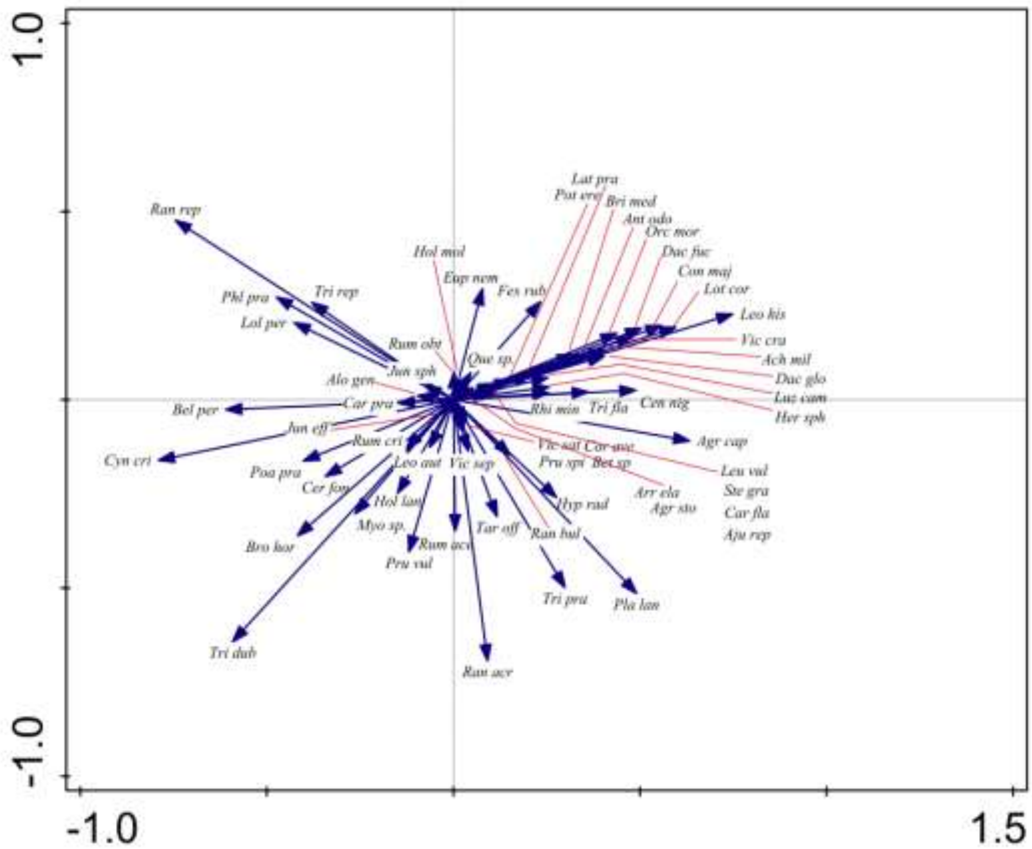


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Figure 5.5.6: PCA species plot of GF source and receiver samples.

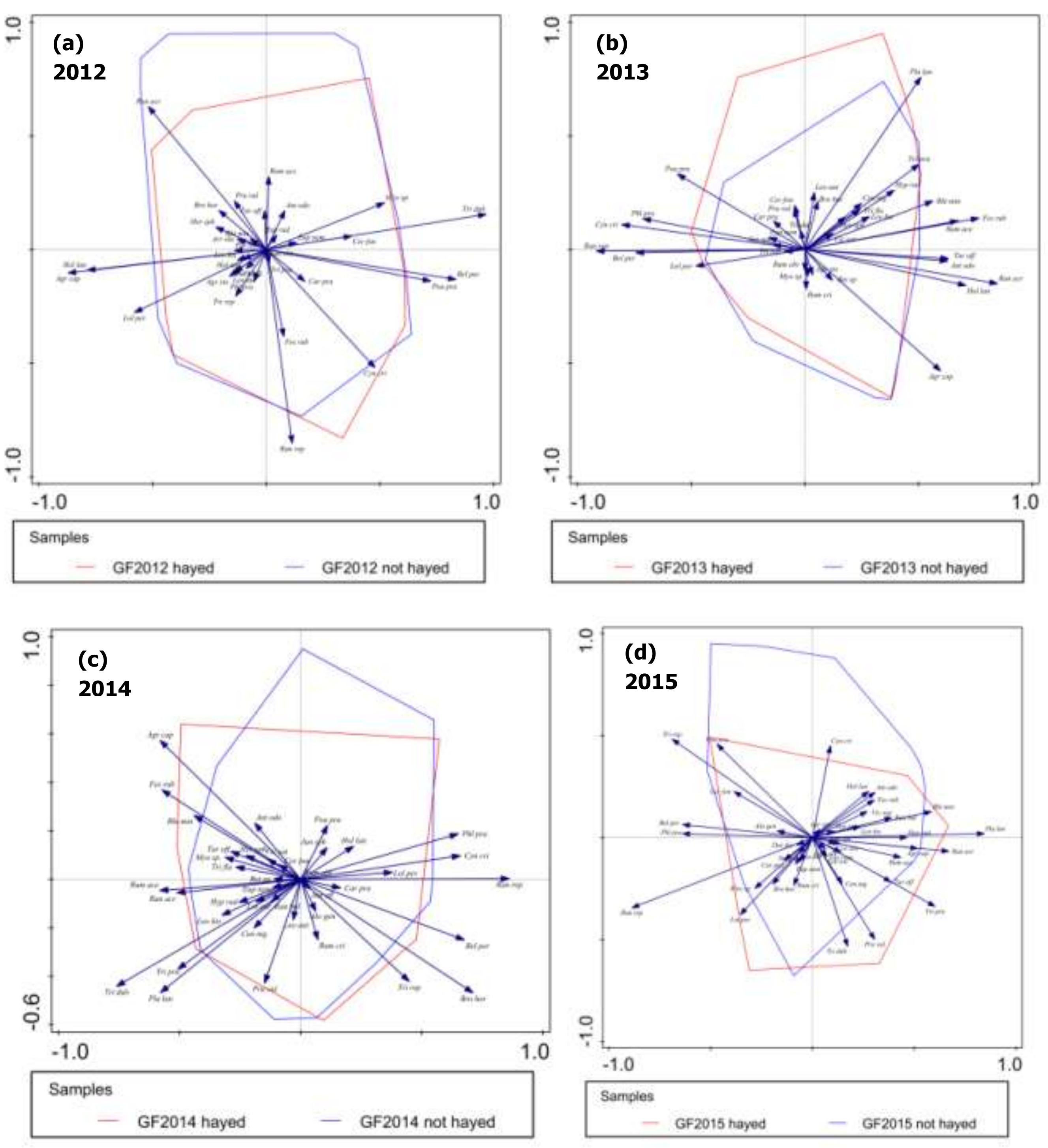


Figure 5.5.7: PCA biplots of the species and samples data for Golden Field, samples coded for haying treatment (a) GF2012, (b) GF2013, (c) GF2014 and (d) GF2015.

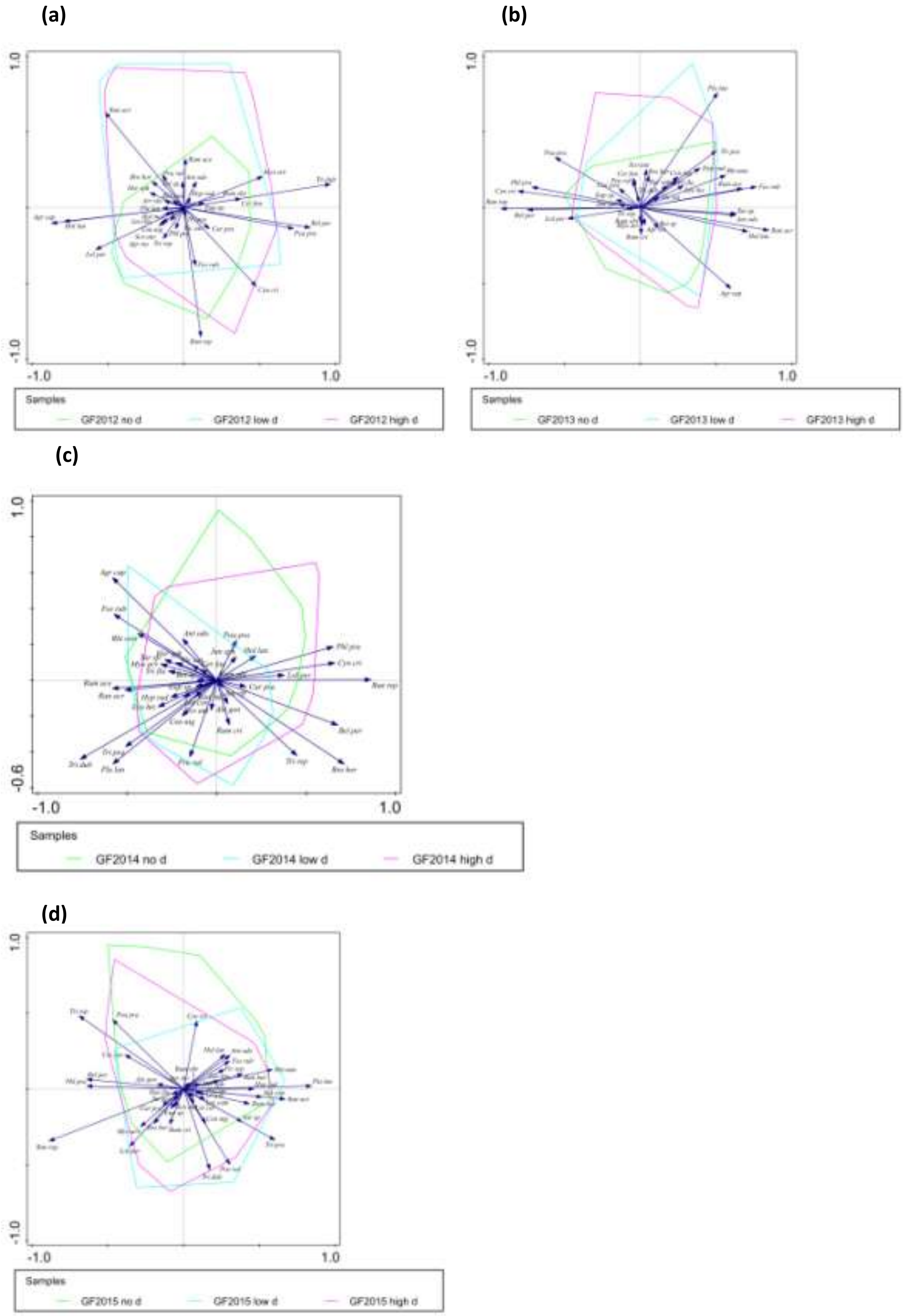


Figure 5.5.8: PCA biplots of the species and samples data for Golden Field, samples coded for disturbance treatment (a) GF2012, (b) GF2013, (c) GF2014 and (d) GF2015.

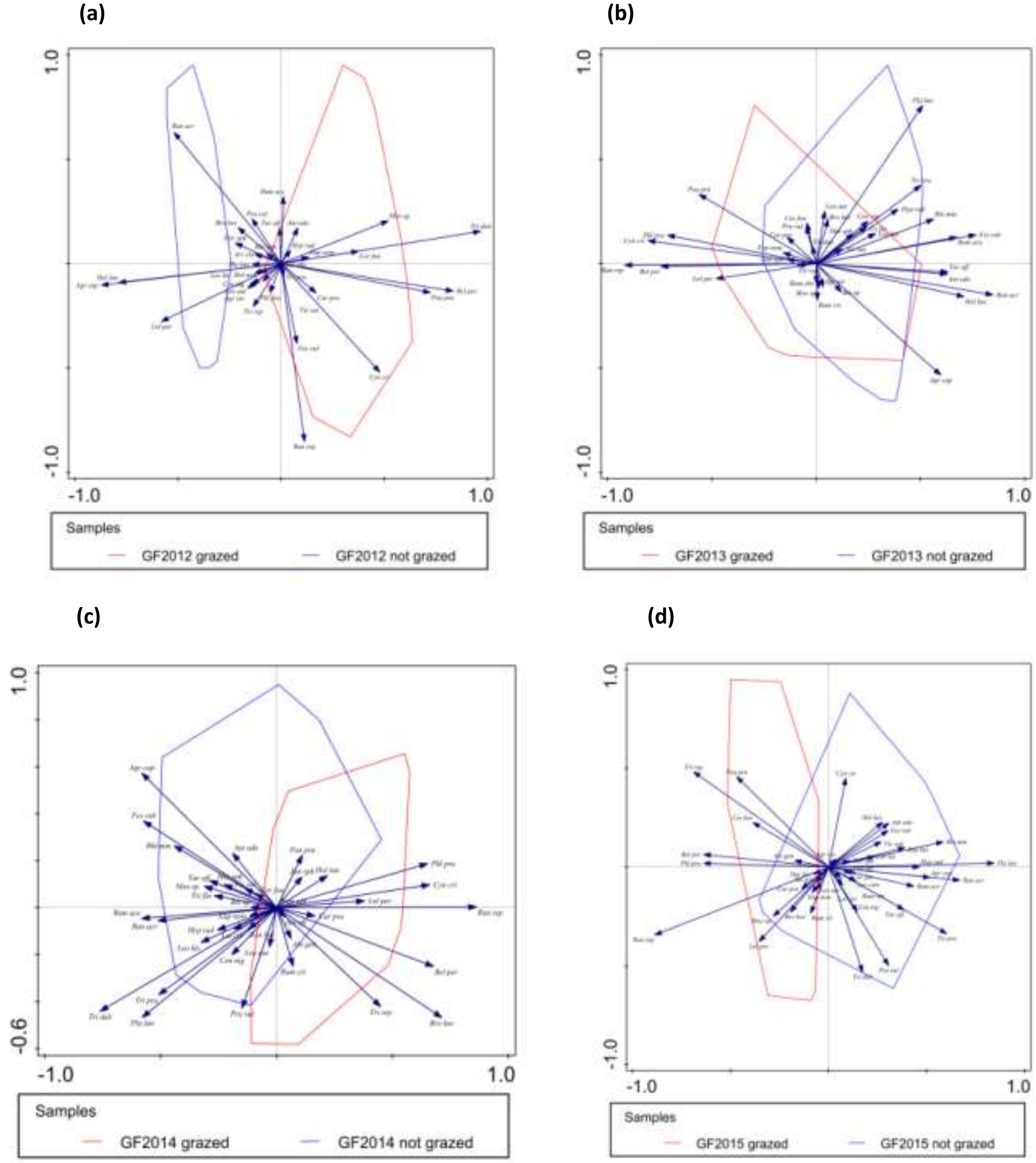


Figure 5.5.9: PCA biplots of the species and samples data for Golden Field, samples coded for grazing treatment (a) GF2012, (b) GF2013, (c) GF2014 and (d) GF2015.

## Appendix 5.6

### TWINSPAN Analysis

Figure 5.6.1 Hierarchical dendrogram of the TWINSPAN analysis for the combined GF2011-2015 and 3YT data.